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PRINCIPLES OF ANIMAL HISTOLOGY

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A TEXT-BOOK
OF THE
PRINCIPLES OF ANIMAL HISTOLOGY

BY

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INTRODUCTION

A TEXT-BOOK of histology must have some very definite reason for appearing at the present time when so many good books bearing that title have appeared in the last few years and promise to continue to appear. These books, however, with practically no exceptions, have been intended for the medical school, and with this in view have been restricted to the histology of man, as a main theme, with more or less reference to other animals, principally the mammals.

Such works have not filled the need of a text-book of general histology for the college course, a book that treats of the principles of the subject. They do not show what the animal cell is capable of as a builder of tissues which enable the organism to make use, more or less completely, of nearly all the known forces of physics and many of those of chemistry. This can be realized when we consider that they do not even mention such tissues as produce electricity, light, gases, and many other things. And again much is lost in the college course in histology by the fact that, of those tissues which are treated of in the medical histology, the complete significance is lost by not seeing their earlier representation and variation in the lower forms.

This volume has been written to secure a work that covers the general field of histology and is not restricted in the main to human and mammalian forms. It is intended to be a work that teaches general principles and teaches histology as a pure science and for its own sake. It is believed that it will serve as a broad foundation for future studies of morphology and embryology as well as for the medical studies.

As to method of treatment, it has seemed convenient to treat each part, with some exceptions, by writing a general discussion of the subject, and following this discussion with detailed laboratory descriptions of types of the tissue, abundantly illustrated with good pen and ink drawings. Such concrete examples have been selected from readily accessible materials in most cases, and the proper procedure for securing and preparing the materials has been indicated in small type at the end of each chapter in many cases. In the last part of the book a chapter on technique will be found, giving a short statement of principles and a guide for some concrete practice. In some chapters the seminar work is not separated from the statements of principles. Many instructors will see the ad-

vantage of giving the student closely allied but different examples to work out with these descriptions.

The work is primarily a treatise on animal histology. Yet some of it is due to researches that have been carried out on plant tissues. In several places fundamental facts are illustrated by materials taken from the vegetable kingdom.

The arrangement and order of presentation of the matter have been given much thought. It has been suggested by many that the arrangement have some relation to the ontogenetic development of the animal body and that the classification of the tissues be based upon their origin from the dividing oösperm. While the many advantages of such a treatment have been fully realized, the writers have felt that a really fairer and more logical method would be an arrangement on a basis of *function* in the first place with ontogenetic and a possible phylogenetic origin treated of necessity in the second place. We believe that such a direct treatment is not only more convenient and clear, but that it is more logical and true, and that it will serve the better to correlate the student's conceptions concerning homology and analogy and kindred relationships.

Perhaps the principal practical objection to the use of the embryological idea in the arrangement of this volume would be the great differences that exist in the fundamental facts of cell-lineage and histogenesis among the principal groups of animals. So long as the work dealt with the vertebrates alone, this difficulty would not emerge, but in a really broad treatment on this basis of almost any principal tissue, much useless repetition and lack of unity would be encountered. The great value of treating the subject from the view point of function can better be understood when it is remembered that *all structures exist only for the purpose of performing certain functions in some particular way.*

The embryological method has not been slighted, however, in arranging this course. It either parallels, or even supplants, at a number of points, the arrangement we have adopted, and it is given a fair if secondary exposition throughout the work. This, as well as other arrangement, has necessitated some small repetition of certain ideas. The writers have not hesitated to repeat leading ideas when it was needful and possible, at the same time, to look at them from different points of view.

A full bibliography has not been inserted in consideration of the fact that the book is intended for college students, and it was thought that a long list of articles, some of them old and others inaccessible, would tend to discourage any further reading. From one to three broad and modern articles by recognized authorities have been mentioned after each part, together with the best general reference books. If the student is to go farther into the subject, the instructor should accustom him to looking up references in the "Zoologischer Jahresbericht" of Naples,

the Journal of the Royal Microscopical Society, and "Schwalbes Jahresbericht" of Heidelberg, as well as in the Zurich Index or Zoölogical Record. He should also be instructed how to find the latest work on a subject that he is seriously studying or is carrying out research work upon. Also to find out who is doing such work or is interested in it.

The book does not pretend to stand as an authority or court of last resort for the specialist in matters of general histology. In most cases it does not carry the student into debated ground. It does attempt to be a convenient teaching guide, to gain the interest of its readers and to stimulate original thought. It is based on the course in "General Histology" given in Princeton University by the senior author during the last ten years.

The writers have many to thank for favors and help in connection with the work. Our colleagues in both universities have often aided us. Dr. H. E. Jordan has made drawings for, revised, and written parts of the work on the reproductive tissues. Professors A. H. Tuttle and Wm. Libbey have read part of the text.

We wish to thank the United States National Museum and the United States Fish Commission for some rare materials given through the courtesy of their officials. Also the Carnegie Institution for much material collected at the Tortuges Laboratory through the kindness of Dr. A. G. Mayer. The writers are most appreciative as well as proud of these American institutions, which are of such continued aid to science.

Mr. and Mrs. Alfred Mitchell most generously provided the opportunity to collect valuable material in Jamaica, which is rich in many rare forms. Dr. F. R. Lillie of Chicago sent us *Unio* material at a time of the year when it could not be collected, and the Yale Museum furnished us with some rare Schizopods.

We wish to extend special thanks to Mr. Charles A. McAlpin of Princeton for his great help in providing the Zoölogical department of the university with quantities of new and old books, back files of periodicals, and other literature. His generosity is one of the factors which has made it possible to do this work in Princeton.

HISTOLOGY

CHAPTER I

PROTOPLASM

An outline of the discoveries which have led us to our present views of life phenomena would not have the logical sequence of the method by which these same facts can be unfolded to the student of to-day. Knowing first only the axiom that living things lived, attention was first drawn to the hollow cell-like structure of plants; and animals were supposed to be likewise constructed. Following this, we came to know the more important fact that a semifluid content of these cells was a specific substance and was the fundamental life-material in living beings. *Protoplasm* was the name given this substance.

Working forward again, we learned of the organization of this protoplasm into the *protoplast* or solid living occupant of Schleiden and Schwann's "cell," with which its name is so hopelessly entangled that we must drop the better term here and use the hollow title of "cell" for the life unit from this point on.

Science was now free to branch out into many wide fields and with much correction of error and understanding of truth to build up our present accumulation of facts which, when compared with the great truths we feel must really exist, is very small. That these truths hinge primarily upon *protoplasm*, and consequently upon the *cell*, the *tissue*, the *organ*, and lastly upon the *individual*, gives us an assurance of the fundamental character and importance of our study of histology which deals with the first three of these fields.

Protoplasm, then, is our beginning, and we find first that it is present in all life forms and that the life manifestations occur through the working of its substance only. It is a transparent, viscid material to the sight and touch and makes up a considerable part of a plant or animal body; not all necessarily, as more or less of such a body is built up of other and non-living substances, which, however, are controlled entirely by the living protoplasm.

Of next greatest interest is perhaps the question, What is protoplasm composed of chemically, and how is it put together structurally? A qualitative analysis shows us that four out of the many known elements

of our universe are invariably mostly concerned, — carbon, oxygen, hydrogen, and nitrogen; and that secondly, smaller quantities of sulphur, phosphorus, iron, etc., are found, some of them only occasionally, in its mass. The four main elements occur in apparently constant quantities: carbon 45 parts; oxygen 28 parts; hydrogen 8 parts; nitrogen 15 parts; and phosphorus 4 parts, which leads one to suppose them united

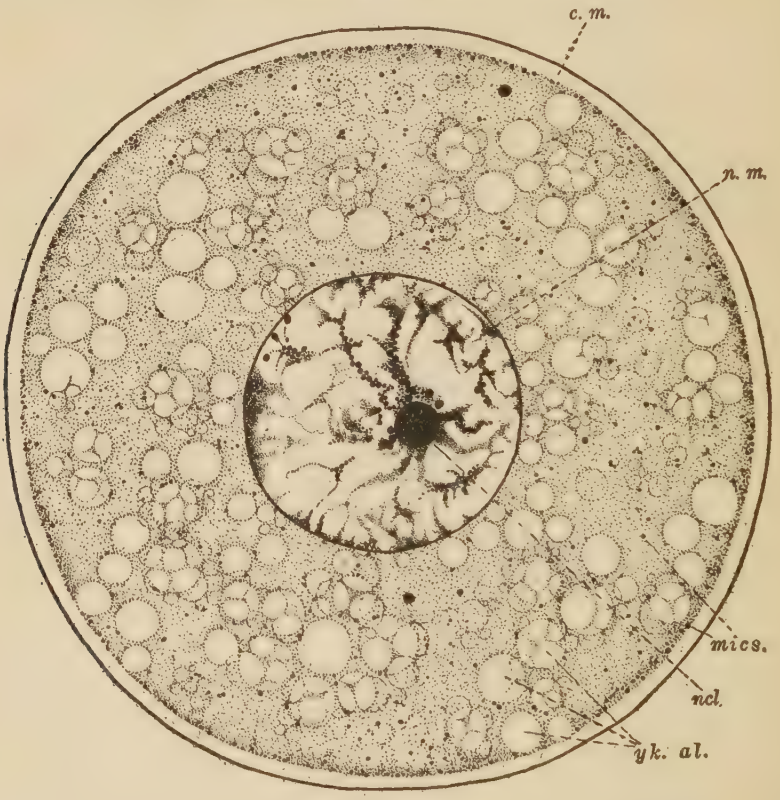

FIG. 1. — Ovarian ovum of a cat just before maturity. *c.m.*, cell membrane; *n.m*, nuclear membrane; *ncl.*, nucleolus; *mics.*, microsomes; *yk.al.*, yolk alveoli.

in a chemical formation in which the second group of elements apparently do not take direct part except, perhaps, the phosphorus.

Turning for light to the structure, it is seen that the protoplasm in a given protoplast or cell is not a homogeneous mass; it is differentiated into several organs of a considerable size, as a nucleus, centrosome, plastid, nucleolus, etc. (Fig. 1), while in finer structure it is evidently composed of several, at least two or three, substances arranged in some sort of a tissue of threads, network, or alveolus, mixed with granules. This finer structure has been described by many investigators as either

alveolar like a mass of foam (Fig. 2) or reticular like a sponge (Fig. 3). In either case this assumes the presence of at least two substances of different qualities. Among these qualities it can be perceived that one substance is of denser and firmer structure than the other, which is fluid. This denser substance, called *spongioplasm*, forms the matrix according to the alveolar theory, or the reticulum according to the reticular theory. Thus it is a continuous and communicating mass in both cases, while the fluid material called the *paraplasm* is considered to be a continuous and communicating mass in the reticular theory only, and is isolated in bubble-like portions according to the alveolar theory. Granules of a much denser material are found scattered about in the reticulum or matrix. They are called *microsomes*. The alveolar theory is the more probable, at the same time admitting that in many cases and at certain times a thread formation of more or less extent does exist, its threads lying in the inclosing substance of the alveolar matrix.

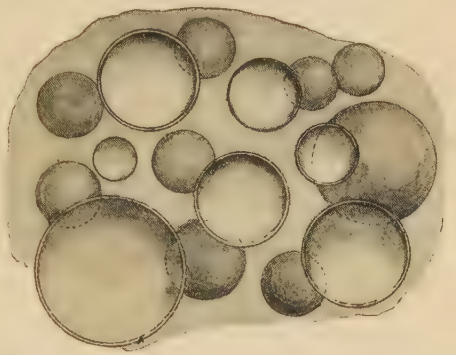

FIG. 2. — Diagram to illustrate the *alveolar* theory of protoplasm.

Knowing, then, that protoplasm is differentiated structurally in the cell, we are prepared to hear from the chemists again, who tell us that protoplasm is not a definite chemical entity, but a combination of several chemical compounds united in a physiological alliance, and interacting on one another in such a manner as to produce the phenomena which we take as evidence of existing life. Passing by some of those substances whose position is doubtful, we can say with probability that those of the compounds known as the proteids are the principal figures in this alliance and the seat of real life. Though composed of nearly the same materials, they are of very great variety, due to differences in the method of composition of these materials. Several of them are usually concerned in the structure of any given

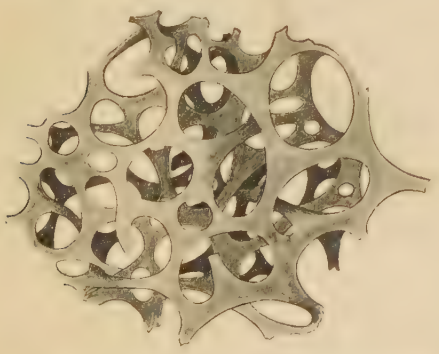

FIG. 3. — Diagram to illustrate the *reticular* theory of protoplasm.

cell, while different cells belonging to different forms of life have each their own peculiar proteid or proteids.

When we realize that these proteids are very changeable and that they go through extensive change upon death, we can understand the difficulty of their study and why we know so little about them.

The questions concerning the exact nature of life lie bound up in protoplasm, and but one of them will be commented upon here. Behind these phenomena of life is there any law or principle other than the present known laws of chemistry and physics that is responsible for the manifestations of the life phenomena? It is our belief that there is, for in no other way can we conceive of the maintenance of so many delicate and variable phenomena for so long a period through so large a number of different conditions.

That many of the life phenomena can be shown to have a direct sequence to some chemical or physical conditions does not convince that such chemical and physical conditions are the first cause of the phenomena; and the fact that life is only maintained within certain chemical and physical limits and conditions again does not show that even such conditions are capable of maintaining it for even a short time. The mathematical law of probability and chance would alone convince one of the futility of trying to make matter live for an instant upon such a basis, when so many conditions are constantly interacting and dependent upon one another. As to the nature or origin of such a "vital" law or set of fixed principles, we know nothing. That such a law or laws are supernatural we deny on the ground that any law once established and continued with the probability of permanence in nature is as natural as any of the laws we know about. That this unknown "vital" law is permanent and is subject to rigid continuance without lapse or exception is the only ground on which it can be discussed. We have not even learned enough of its manifestations, as yet, to in any way define it or to classify its results other than to believe that it directs and controls the life processes to ends which vary only with the circumstances under which they exist.

Protoplasm is protoplasm only so long as it is living. As before mentioned, life must be regarded as a process that is taking place constantly in the protoplasm. It is a complicated process which results in constant change and requires a constant supply of new material or food. As a result of this activity, energy is produced in the form of motion, heat, light, and electricity; also many materials are elaborated, as acids, carbohydrates, digestive fluids, protective and supporting materials, poisonous and offensive substances, etc., which are necessary to the existence of the creature to which the protoplasm belongs.

The dynamic products of protoplasm — heat, light, electricity, and

motion — are not produced directly by the living material itself but by the chemical activities of substances that the protoplasm has formed by its own "vital" activity, in the same way that it produces the ferments, acids, and other substances mentioned above.

The chief difference seems to be that the latter are discharged from the cell after secretion to be used in other places, while the heat, light, and electricity producing granules are used inside the cell (light granules or *photochondria* are sometimes used outside).

By secretion we shall always mean the elaboration of material in the protoplasm by the activity of the latter. The ejectment of this material by the cell or the gland of which it is a part will be termed *discharge*. Mathews has called the secretory process *hyalogenesis*, and the particles so formed *hyalogens*. This is done to avoid using "secretion" in the sense that we use it, as he believes this word to mean what we mean by "discharge."

The above discussion reduces us to the idea that the cell can produce only a *substance* or material. The method of producing these substances is practically not known at all, and the directive force that controls the activities of the cell is entirely unknown and a subject for crude speculation.

Technic. — The most ordinary methods carried out with the greatest care are the best to use in the study of protoplasm. Flemming's fluid, paraffin sectioning, and iron hæmatoxylin staining will probably give the truest pictures. Living material, as the bodies of undifferentiated Protozoa, etc., should also be studied under a very moderate pressure. These latter must be examined under an immersion lens with the diaphragm reduced to get the best optical results. A particularly beautiful picture of protoplasmic structure may be obtained by cutting well-fixed material in celloidin, and either staining in bulk or after sectioning. The lack of shrinkage in such sections, and the fact that one sees a deeper layer than in the very thin paraffin sections, secures a picture that should be studied in connection with the paraffin preparations. One should also examine the tissue alive in salt solution. Pressure is, then, sometimes necessary to get a thin enough layer to work with.

SOME LITERATURE ON PROTOPLASM

BÜTSCHLI, O., 1892. Untersuchungen über mikroskopische Schaume und das Protoplasma. Leipzig, Wilhelm Engelmann.

WILSON, E. B., 1899. "The Structure of Protoplasm," *Journal of Morphology*, XV.

CHAPTER II

THE CELL

Protoplasm, as it is ordinarily encountered in living things, is always organized as certain structural units in which it shows some characteristic differentiation. **The cell is such a working unit of protoplasm** (see Fig. 1). These living protoplasmic units are the structural units of which all organisms are formed. Living units of an extremely low type have been described, which consist of simple undifferentiated protoplasm. These forms are not very frequently encountered, and it is probable that the lack of differentiation is due to the inefficiency of the methods employed in their study.

Fig. 4. — *Tetramitus chilomonas*, a unicellular animal which shows the nuclear material in a distributed condition; *chr.*, chromatin masses. From Calkins.

The simplest of these cells are represented in some low forms of plants and animals (Fig. 4). In these low forms a certain differentiation of the protoplasm is detected by staining processes. Scattered throughout the general protoplasm of such a unit are numerous rounded granules which stain deeply, while the remaining substance does not stain. The granules, because of their affinity for stain, have been called *chromatin*. The non-staining part of such a structural unit is spoken of as *cytoplasm*. A structural unit or cell of this character, which has chromatin diffused or scattered throughout its cytoplasm, is called by some a *pseudocyst* or *false cell*. Figure 4 shows such an animal in which the chromatin appears distributed throughout the cell.

Except for these low forms of life, the unit of structure is always a more highly specialized mass of protoplasm. Part of the protoplasm of these higher cells has been differentiated to form a specific mass that always is found within the less highly specialized protoplasm. This is known as the *nucleus*, and the protoplasm within which it lies receives the term *cytoplasm*. The nucleus itself is clearly defined as a round to irregularly shaped body, more dense than the cytoplasm. It is by no means homogeneous. The modified protoplasm or *karyoplasm* of which

it is composed has undergone further differentiation, as may be seen in Figure 1.

The nucleus is clearly defined from the cytoplasm by a film of specialized karyoplasm which has generally been looked upon as having a membranous texture and has, therefore, received the name *nuclear membrane*. The nature of this membrane has not yet been satisfactorily determined. The nuclear membrane incloses a transparent, refractive fluid, the *hyaloplasm*.

Supported by the hyaloplasm is a network of non-staining refractive threads which form a scaffolding upon which other karyoplasmic structures are distributed. These threads are composed of the *linin* of the nucleus.

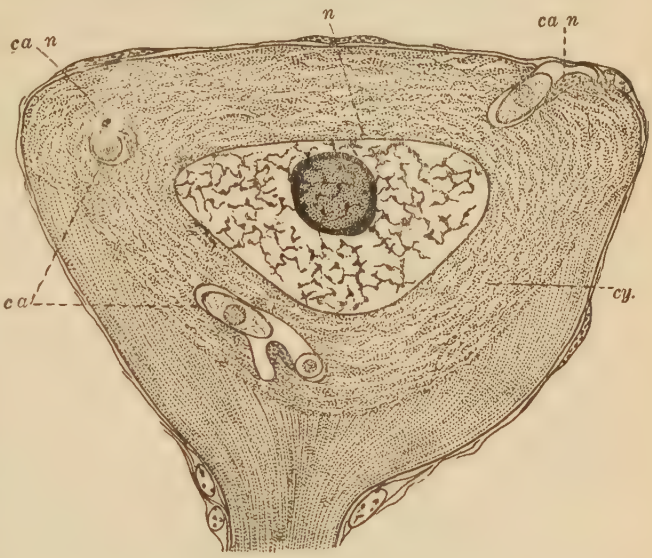

FIG. 5. — Dorsal nerve cell from cord of *Pterophryne histrio*. *ca.*, blood capillaries containing blood corpuscles; *n.*, nucleus of nerve cell; *ca.n.*, nuclei of capillary wall; *cy.*, cytoplasm. × 400.

Tangled within the linin meshes are usually to be seen from one to many rather large, rounded bodies which stain more readily than the linin and are relatively dense and highly refractive. These are the nucleoli or *plasmosomes*. Their form varies. In some instances they are rod-shaped, in others they become angular lumps. Their texture is homogeneous. They frequently inclose vacuoles (Fig. 5).

The most essential parts of the karyoplasm are the deeply staining granules which are supported upon the linin network. These are quite probably the homologues of the deeply staining granules of pseudocysts, and they have suggested the name for the latter, they having been called *chromatin*. The chromatin in a cell that is not dividing is distributed through the nucleus in a number of particles of irregular shape, as in Figure 1. These chromatin grains are usually many and are clearly defined; but they may be either few and relatively large or so extremely minute that they are individually invisible and give a cloud-like appear-

ance to the nucleus. Sometimes certain portions of the chromatin are in the form of bodies that greatly resemble nucleoli (Fig. 6). Although such chromatin bodies and nucleoli both stain deeply, the shade and quality of stain show them to be different. The nucleoli or plasmosomes

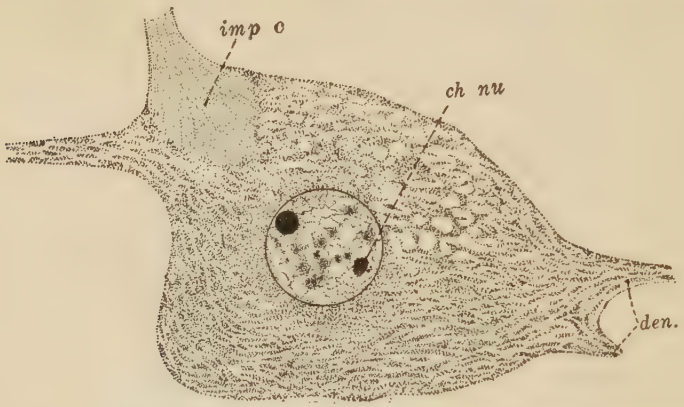

FIG. 6. — Motor nerve cell from electric lobe of brain of *Tetronarce*. *imp.c.*, implantation cone and beginning of neurite; *den.*, dendrites; *ch.nu.*, chromatin knot. × 1500.

stain less intensely than the chromatin masses. These chromatin masses are called *karyosomes* or chromatin knots (see Fig. 6). Being composed of a number of individual chromatin grains, they are rough and irregular, while the plasmosomes are smooth in outline. The nucleus sometimes contains another structure, the *centrosome*, which, as it is usually found lying outside the nucleus in the cytoplasm, will be described in connection with the cytoplasm.

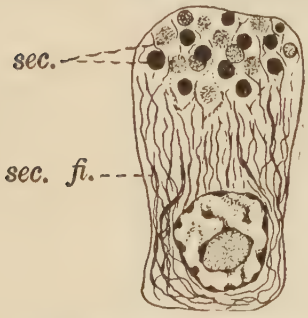

FIG. 7.—Pancreas cell from salamander. *sec.*, secretion substance; *fi.*, secretion fibrils. After MATHEWS.

The cytoplasm may be looked upon as the least differentiated part of the cell of higher organisms. The general study of protoplasm has mostly, if not always, been based upon the cytoplasm. Hence what was said in Chapter I of the texture of protoplasm will apply to cytoplasm. It usually presents an alveolar appearance. The alveoli may be so small that the cytoplasm appears not to be alveolar. Numerous minute granules always form part of the cytoplasm. They are the *microsomes* (see Fig. 1). Fibrillæ are frequently present. They are to be considered as differentiated cytoplasmic structures which have to do with certain activities of the cell. They seem to be closely associated with certain forms of cytoplasmic activity, with secretion and excretion,

and nuclear division (Fig. 7, and see Figs. 34 and 137). The cytoplasm is the vegetative part of the cell and is frequently charged with foodstuffs, such as yolk granules and starch, and with secretion and excretion products (Figs. 8 and 9). *Plastids* and *chloroplasts* are found most frequently in plant cells (Fig. 10). *Vacuoles* containing fluids are frequently found in animal cells and are always present in a mature plant cell. Within the vacuoles, crystals and other solids are sometimes stored (see Fig. 9).

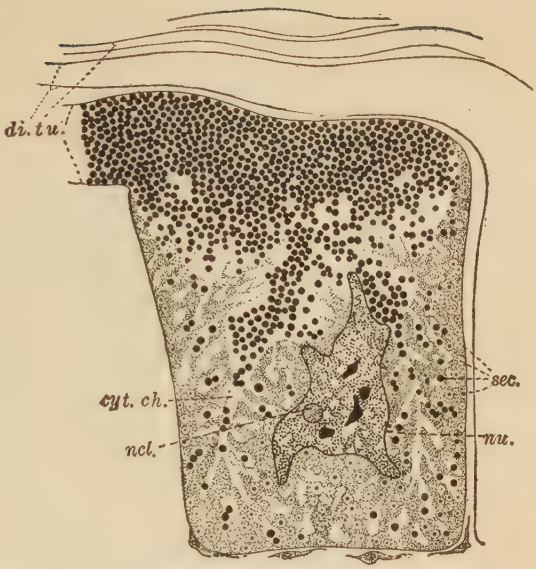

FIG. 8. — Gland cell from leech, *Pisicola*. *sec.*, secreted materials in various stages of elaboration; *ncl.*, nucleolus; *nu.*, nucleus; *cyt.ch.*, cytoplasmic channels containing and delivering the secretion granules to the large distal vacuole; *di.tu.*, discharging tubes of this and two other cells.

A very important structure is sometimes found in the cytoplasm as a permanent feature; sometimes it is but a temporary structure, arising *de novo*, and in other cases it may be absent.

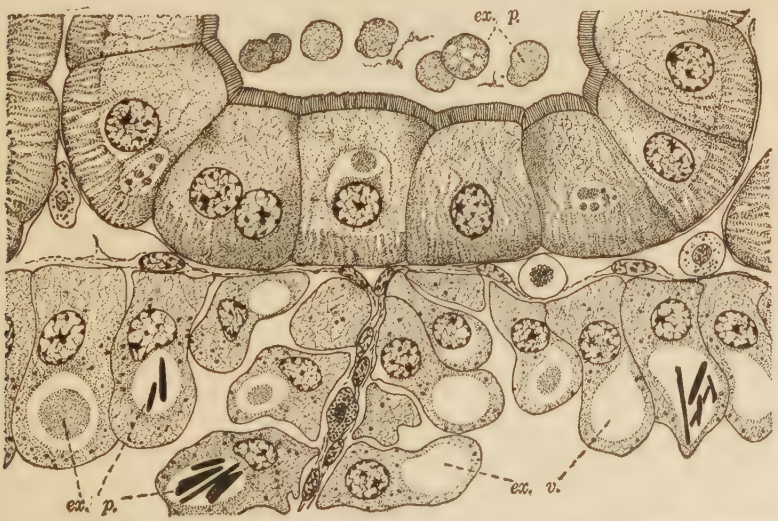

FIG. 9. — Section through parts of two regions of lobster's nephridium. *ex.p.*, excretory products; *ex.v.*, excretory vacuoles. × 425.

This is the *centrosome* (Fig. 11). It consists of a number of radiating fibers, which may be absent when the centrosome is quiet; they form the *aster*. These fibers converge about a dense area in the cytoplasm, the *attraction sphere* (Fig. 11). Within the attraction sphere there are present one or more, usually two, minute bodies called the *centrioles*.

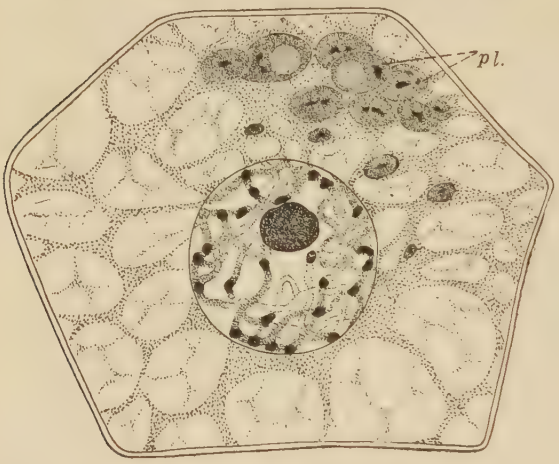

FIG. 10. — Cell from root cap of calla lily. *pl.*, plastids. × 700.

In a living cell, the cytoplasm and the karyoplasm or nucleus are vitally related. The nucleus is influenced by the cytoplasm. Different parts of the cytoplasm, indeed, seem to affect differently the dividing nucleus in such a manner as to determine the orientation or position of the nucleus, as has been observed by Lillie in the dividing cells of *Unio* eggs. This gives rise to a definite axis in the cell, and it is then said to have polarity.

Axes of cells are also determined by secretory structures. In epithelial cells of excretory or secretory functions, fibrils and excretion particles appear. These and the nucleus in such cells do not lie promiscuously within the cell, but they have a definite arrangement. The fibrils run from the free surface toward the base of the cell, and in many forms converge to a common point. The elaborated products appear at various levels of the cell and move toward the free surface either bodily as granules, or in invisible solutions. Thus a longitudinal axis is established for the cell. With reference to this axis, cells may be radially or bilaterally sym-

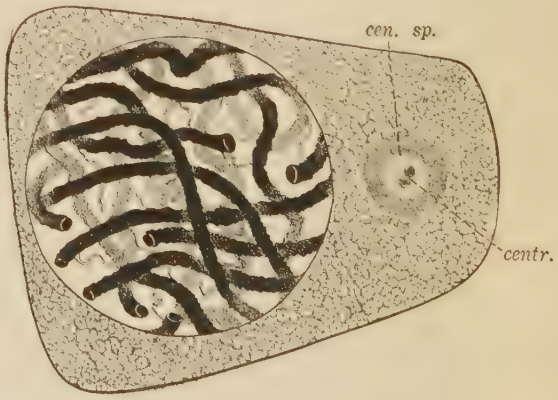

FIG. 11. — Spermatogonium of salamander, *Spelerpes ruber*, containing a centrosome. *centr.*, centrioles; *cen. sp.*, centrosphere (composed of an inner and an outer zone).

metrical. Haidenhain speaks of cells with such polarity as being *dorso-ventrally symmetrical.*

The nucleus cannot live outside of the cytoplasm. In general the nucleus is found lying in the cell where it has the best opportunity for the most extensive and intimate contact between its surface and the main cytoplasmic mass. It usually lies in a central position in the cytoplasm, but it may lie at the extreme periphery. In fact it sometimes lies so far out that it occupies a position outside of the general outline of the cell, and is covered by a mere film of cytoplasm. In turn the cytoplasm cannot live for long or reproduce itself without the nucleus.

In plants the cell is usually inclosed within a cellulose cell-wall. In animal cells there is, as a rule, but an indefinite *cell-membrane;* most of the figures of animal cells will show this. In many tissues the cell-walls or cell-membranes are not present. This results in a blending of the cytoplasm so that the number of individual cells can only be determined by the nuclei; the cell boundaries in such tissues cannot be determined. Such a mass of cytoplasm, with frequent nuclei, is called a *syncytium.*

One of the features of cell organization is size. With very rare exceptions the cell is a very small body only a few thousandths of a millimeter in diameter, and while (relatively) some cells may be twice or ten times as large as some others, yet they nearly always remain microscopic bodies. In the few cases where they are macroscopic objects, as the hen's ovum and certain low plants, we find that this unusual size is due, *not to a larger mass of protoplasm,* but to *non-living contents* or to *internal vacuoles* or *spaces.* The structure of protoplasm evidently prohibits its working in more than a certain sized mass, nor is this an arbitrary rule. The interchange of material between cell mass and exterior, which is constantly and necessarily taking place in living protoplasm, would alone give us good grounds for this conception, when we remember the fact that the surface, through which nutritive, waste, and other materials must pass, increases as the square, while the content or mass, which must be supplied, increases as the cube of the dimension. This idea is much strengthened when we remember that most of the very largest solid

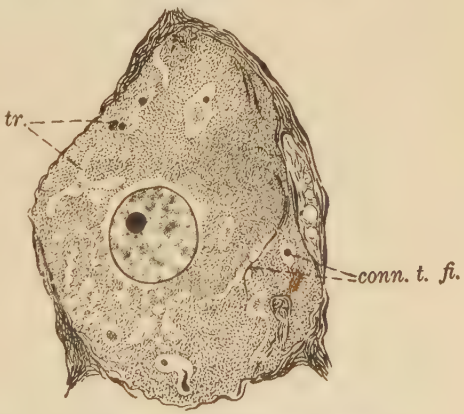

FIG. 12. — Nerve cell from stellate ganglion of squid, *Loligo Pealii. tr.*, trophospongia or intracellular blood channels; *conn.t.fi.*, connective tissue fibrils penetrating the cell, especially by way of trophospongia.

cells known have increased their available surface by invaginations, which form channels that carry lymph, blood, and waste matter. They thus avoid the penalty of their size at the expense of extreme specialization. Figure 5 shows such a cell with a vascular circulation. Other channels are found inside some cells which are used for internal transportation or for the ultimate gathering of fluid and solid secretions and their removal to gland lumina. These have been called *trophospongia* by Holmgren, their discoverer, and they may be seen in Figure 12 as well as other figures.

The shape of the cell varies more greatly than its size: Cells, where free from distorting lateral pressure, are as nearly spherical as possible. Their position and use often cause them to assume very extraordinary shapes, such as flat disks, long rods, and extreme branching forms.

Technic. — The same remarks that followed the part on protoplasm will apply here. To get an accurate conception of the organization of any kind of a cell, it is necessary to not only prepare this cell by a number of the best methods, but to also make many preparations by the same method. These latter preparations will differ much among themselves as to the form of the different organs and the staining powers of the different cell-substances.

Flemming's strong fixing fluid is perhaps the best fixative known in the majority of cases. It requires the most care and skill in its use, but gives the truest pictures of the materials prepared by its agency. Zenker's fluid, chrom-aceto-formol, and picro-acetic are examples of some of the best of the other fluids, and they should all be tried.

LITERATURE

The subject is so extensive and the literature consequently so large that the student is referred to the general text-books. Wilson, "The Cell," Schneider, "Lehrbuch der Histologie," and Hertwig's "Cell" will give extensive reading.

CHAPTER III

MULTICELLULAR ORGANIZATION: PHYLOGENETIC

According to the doctrine of descent, which at present receives wide recognition, all animals have evolved from some simpler forms. It is held that every multicellular animal or plant has developed through an infinite series of stages from a unicellular form. The geological formations bear broken records of such an advance in structure. This past history of a race is called its *phylogeny*. Necessarily it is a history of which we have no human records, since man did not exist during the longer early periods and was not a scientific investigator until a comparatively recent time. Our only actual evidence lies in comparative study of the fossil remains of such creatures as happened to be preserved in the rocks. Of these we have only the hard parts, as bone and shell in most cases, and but few actual histological structures have been preserved, as in the case of some selachian muscle and some crustacean integument.

These fossil remains show that in earliest times only the simplest forms existed, and that, as ages passed, larger as well as more complex forms were added. Some races were entirely lost during these changes. The geological record also shows that the simplest and intermediate groups continued to exist with little change to the present time, except some that have been lost entirely. Therefore, we may assume that the present large series of animals, known as the *taxonomic* series, represents, to a certain degree, the long extinct *phylogenetic* series whose broken record we find in the rocks.

The taxonomic series suggests that higher efficiency in organization has been effected along two lines: First, by an increase in the mass of the individual; and secondly, by a differentiation of the component cells of an individual. How these modifications were accomplished can, to a certain degree, be understood by a study of certain living forms.

We have seen in the preceding chapter that, though the specialization in cellular organization be varied, the maximum size of cells is soon reached. It is only exceptionally that unicellular creatures attain macroscopic dimensions. Increase of bulk is, therefore, rarely effected by the growth of a single cell and is usually accomplished by the

grouping of cells to form a colony. In such colonization of cells we have represented the first step toward multicellular organization. The relation of the cells to each other in these multicellular organisms varies with the degree of advancement the colony has attained. This affords a basis for dividing multicellular organisms into *three orders* of colonization. The cells constituting any one of these kinds of colonies should usually be considered as descendants of a single cell or otherwise closely related.

In a colony of the first order the component cells have no intimate, vital relation with one another. This colonization results in a mere increase in bulk, by which the cells are mutually protected against the disturbing forces of their environment. Each cell of such a colony is capable of living alone, if detached from the colony; and the colony exists with an indefinite number of cells. In this case the cell and not the colony must be considered the individual. Such colonies are found represented by certain Protophyta and Protozoa.

In the second order of cell-colonies we find a more intimate relation existing between the members of the cell aggregation. In a colony of this type the number of component cells is always constant. The death of one or more of the cells must result in their replacement by new cells. In such a colony one cell cannot move independently of the others. This is a comparatively simple mode of colonization. No cell is here concerned in a peculiar manner with the life of the colony, each cell performing all of the vital functions. Algæ such as *Pandorina*, *Eudorina*, and *Gonium* present this type of colonization.

The third and highest order of colonization or cell aggregation presents groups of cells vitally related to one another and in which the cells are not all alike. A certain amount of differentiation has taken place, with the result that certain cells with changed character are set apart to perform definite functions. This is the type of cell-colony met with in all the Metazoa. Phylogenetically this order of cell-colony probably would fall under two divisions. One division would include the colonies in which the differentiated cells remained independent of their fellows which performed the same function. The reproductive cells, for example, were not confined to a particular region of the colony, but were scattered independently throughout the cell aggregate. An example of this simple multicellular organism of the third order may be seen in *Volvox globator*. In the second division of this third order of colonization we meet with a higher grade of organization. In a cell aggregate of this type the cells differentiated to perform a particular function are assembled in a particular part of the body of the animal. The segregation of cells similarly modified has given rise to what is known in histology as a *tissue*. *A tissue, then, is an aggregation of cells that have been specialized to*

perform or help perform a definite function. As an example of an animal belonging to this second division of the third order of multicellular organization, we may cite *Hydra.*

Multicellular organization in the narrower sense of the term ends here; but this does not cover the scope of multicellular organization. Just as cells are assembled to form a colony in which certain of them are differentiated and segregated to form tissues, so tissues have been differentiated and segregated to perform definite functions or sets of functions, giving rise to organs. *An organ, then, is a group of differentiated tissues performing some particular function.*

Multicellular organisms, therefore, range from groups composed of an indefinite number of cells scarcely, if at all, related to each other, to individuals composed of closely interrelated and mutually dependent organs. It is also held that the phylogenetic series was equally as extensive. This is rendered all the more likely by the evidence gathered from the study of the ontogenetic series.

An Example of a Colony of the First Order: *Carchesium.* — The individual cells in a colony of *Carchesium* are attached to a branched system of stalks which they themselves have elaborated. There is, in this system of stalks, a set of radiating branches that arise from a common point of attachment. From these radial branches short lateral branches are given off at more regular intervals. At the end of each radial and each lateral branch a single cell is borne (Fig. 13). Each animal is a bell-shaped cell measuring, when extended, about fifty microns in diameter and seventy-five microns in length. The cytoplasm is differentiated into an *ectoplasm* and an *endoplasm.* Within the endoplasm there is a constant cyclosis. This endoplasm contains a sausage-shaped macronucleus and a single rounded micronucleus. These nuclei are not carried with the cyclosis, but have a stationary position in the cell. Opening out from the ectoplasm is a single contractile vacuole. At the distal end there is a ciliated zone which makes about one and a half spirals as it winds about the body to enter the "gullet." At the base of the gullet the food passes through the mouth into the endoplasm to form a food vacuole. The stalk of each animal is provided with a contractile fiber (Fig. 13, *f.*).

FIG. 13. — A colony of individuals of *Carchesium* attached by a common branching stalk; *f*, contractile stalk of one individual. (REMY after PERRIER.)

Each animal lives at the end of its stalk independently of the others of the body. It secures its food by means of its own ciliary movements and carries on for itself all metabolic and reproductive functions independently of the colony as a whole.

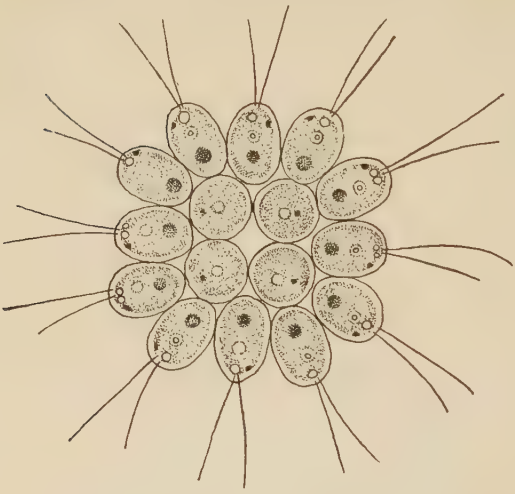

FIG. 14. — *Gonium pectorale*, showing the individuals. (From CALKINS after STEIN.)

An Example of a Colony of the Second Order: *Gonium pectorale*. — A specimen of *Gonium pectorale* is always composed of sixteen oval cells attached laterally to one another in such a manner as to form a square colony with twelve cells on the margin of the square and four cells inclosed by the lateral ones. The entire colony is surrounded by a gelatinous sheath. Each cell is oval and inclosed within a cellulose wall. The cytoplasm contains a cup-shaped green chloroplast and a centrally placed nucleus. Near the open margin of the chloroplast there is a bright red stigma. In the cytoplasm opposite the opening of the chloroplast are two contractile vacuoles. From this same region of the cell two slender flagella are given off (Fig. 14).

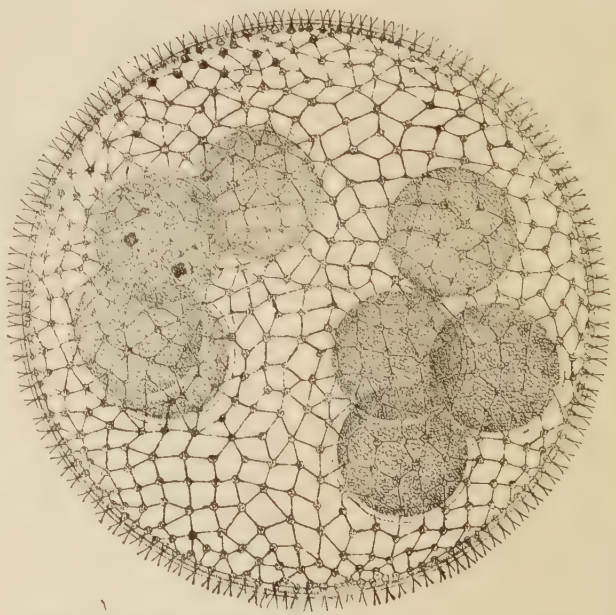

FIG. 15. — *Volvox globator*, a colonial organism of the third order. (From WILSON, after J. H. EMERTON.)

It is to be noted in this connection that here no cell has the power to move independently of its fellows.

An Example of Colonies of the Third Order; First Division: *Volvox globator.* — This creature is spherical, with all the cells confined to the surface of the sphere so that the sphere has a cellular wall and a cavity bearing no cells. A *Volvox* colony of ordinary size measures three hundred microns in diameter. There is no definite number of cells in a colony, so that the size varies greatly; a colony may measure over seven hundred microns or even a thousand microns in diameter. Each cell has all the parts described as belonging to a cell of *Gonium pectorale.* The cells are united to each other by protoplasmic strands radiating from each cell (Fig. 15). The colony revolves on a definite axis, and moves toward one of its poles. The stigmata, which probably have to do with the receptions of light impressions, are, therefore, always located in each cell to face in the direction of travel as much as their position in the cell-colony will permit. It is of chief importance here to note that certain cells are modified to perform sexual functions. Any cell of the mass may become a female cell or give rise to a group of male cells. The female cell is a large spherical cell free from flagella and provided with a large nucleus and a highly granular cytoplasm. This cell is called an ovum (see Fig. 15). Our figure shows the primitive germ cells. By repeated division, certain of these germ cells become separate clusters of small, colorless, spindle-shaped cells with two flagella. These are the male cells or *sperm-cells.* It is to be seen here that the ova are distributed promiscuously throughout the vegetative cells. There is apparently a tendency to assemble cells differentiated in a particular manner seen in the groups of sperm-cells; but this is only due to their mode of origin. The clusters of sperms arising from

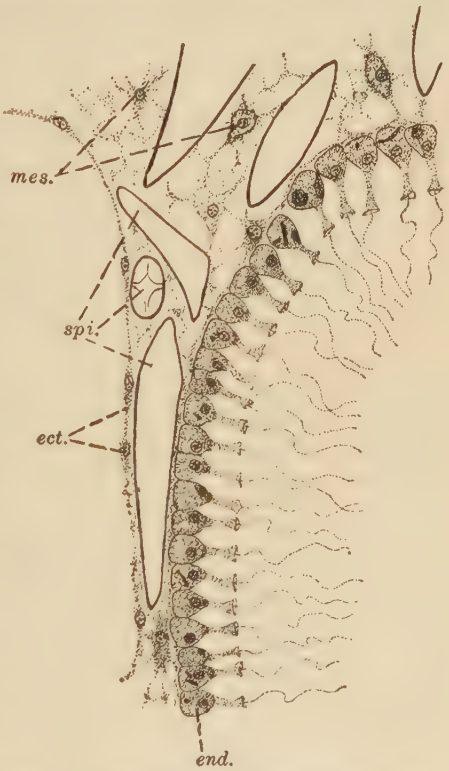

FIG. 16. — Part of the body of a sponge, *Grantia: mes.*, mesoderm; *ect.*, ectoderm; *end.*, endoderm; *spi.*, spicules.

widely separated cells are not assembled to form a definite region in the colony.

Second Division: *Grantia sp.*— In *Grantia* and other sponges we meet with a triploblastic colony; by this we mean a cell aggregate composed of three layers of cells. The outer layer of cells forms a thin layer of nucleated cytoplasm (Fig. 16, *ect.*). The inner layer is composed chiefly of oval cells supplied at their free ends with a bell-shaped collar and a slender flagellum (Fig. 16, *end.*). Between them lies a layer of greatly branched, anastomosing cells. These cells bear rounded nuclei (Fig. 16, *mes.*). We observe here that the cells modified to perform the function of protection are assigned to a particular region of the cell-colony and that they form a continuous outer layer, the *ectoderm* (*ect.*). Likewise the cells performing the function of alimentation are assembled and relegated to particular regions to form part of the *endoderm* (*end.*). Finally, between these two tissues we note the third tissue made up of cells looking chiefly after the mechanical support of the colony and uniting to form a loose tissue — the mesoderm (*mes.*).

LITERATURE

Read general articles and parts of such books as "The Foundations of Zoölogy," by W. K. Brooks.

CHAPTER IV

MULTICELLULAR ORGANIZATION: ONTOGENETIC

THE developmental history of a multicellular animal from the egg to full maturity is known as its *ontogeny*. The soma or body of such an animal is thus an aggregate of cells descended by successive divisions from a single cell, the fertilized ovum or *oösperm*. These cells form connected masses, resulting in one or more bodies or individuals, and in such an individual it will be noticed that two things have occurred.

First. The cells, during their successive generations, have grown to be of several different kinds, each of which is adapted to perform some particular function that the individual may be called to maintain, and,

Second. These different kinds of cells have been grouped apart or together to form regions and relations with each other that will best permit them to perform their peculiar functions. Such regions or associations of cells, together with their products, are called *tissues;* or, where certain tissues are very especially arranged apart from other tissues, the tissue aggregate is known as an *organ*.

These two conditions are known as *differentiation* and *organization* respectively, and the ability of the creature to maintain its life and place in the world depends upon the degree of efficiency which differentiation and organization have attained in relation to the conditions under which the creature exists.

One of the most important differentiations occurs early in the life history and represents the separation of all the cells that are to undergo further differentiation from one or more of them that do not differentiate fundamentally but remain as a store of the original material, to be used later in building up other organisms of the same kind (see Chapter XXI). These latter are called the *reproductive cells* (Fig. 17). They undergo a very special differentiation of their own at their time of maturity. They may be many or few, and some of them sometimes appear to not only retain their original reproductive powers, but to also perform, in a degree, some of the body functions. These may be considered, therefore, under such circumstances, as somatic cells. In this case we have a slight differentiation as compared with the higher forms

in which the somatic cells are so strongly and so early differentiated from the reproductive cells that they cannot retain their power of reproduction and perform somatic duties at the same time.

In the first examples the groups of somatic cells are usually also slightly differentiated from each other, while in the latter they are highly differentiated.

This differentiation does not all come about at once. The dividing oösperm may transmit to its descendants all of its qualities and original powers and structures for a considerable number of cell generations before any one of these descendants begins to differentiate. Or the dif-

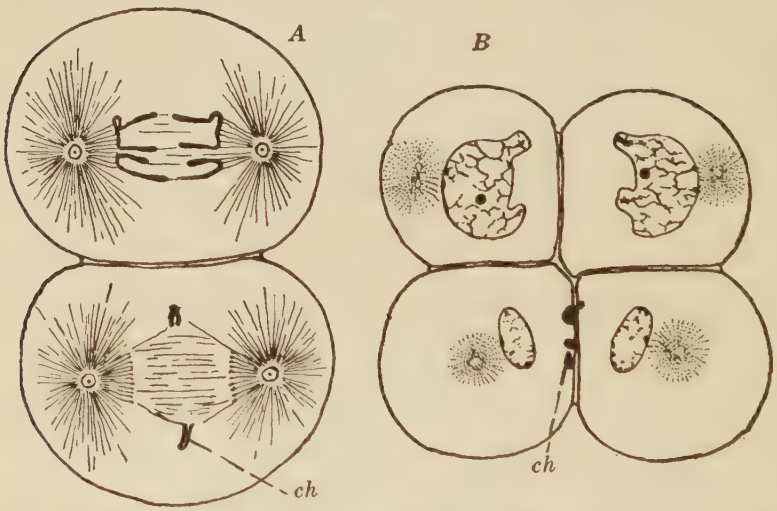

FIG. 17. — *A*, second cleavage division of the oösperm of *Ascaris*, showing the first differentiation by loss of chromatin in the somatic cell. *B*, resulting four cells, showing the lost chromatin, *ch.*, and the smaller resulting nuclei in the daughter somatic cells. (From WILSON after BOVERI.)

ferentiation may begin by changes in one or the other of the two cells produced by the first cleavage division. In fact the frequent greater size of one of these first two daughter cells of the oösperm shows that there were differentiating forces in the oösperm itself before it began to divide, and we are thus brought to see that the beginnings of differentiation are sometimes *preformed* in the oösperm. This can be seen in a number of ways in the developing eggs of several kinds of animals and may be discussed under a few principal headings.

The simplest and most fundamental form is that seen in the organisms whose oösperms show a distinct polarity in their organization, as in the frog and many other animals. A feature of this polarity is the collection of the yolk or food supply of the ovum at its lower end and of the

chief fundaments of its future nervous, muscular, and other organization at the upper pole.

This might not appear to be so primitive a distinction as the early evidence of bilateral symmetry seen or inferred in the oösperms of other animals were it not for the fact that it may be compared functionally with the early taxonomic associations of individual cells mentioned in the preceding chapter and serving here as a suggestion of phylogenetic history. In these, the use of the lower surface of the mass for the acquisition of food may be compared to the storing of food in the lower part of the body of the dividing ovum and to the subsequent development of this surface by invagination into the chief digestive organs of the body. This invagination of the lower body surface is known as gastrulation. By this process the body mass of the young embryo becomes arranged in an upper and a lower layer which are called respectively the *ectoderm* and

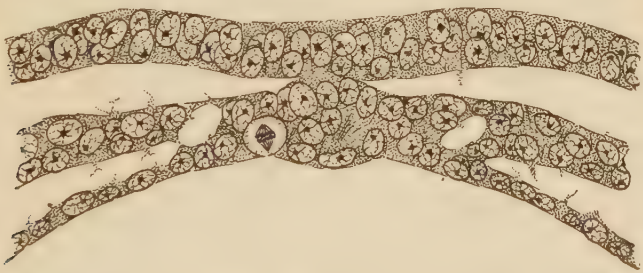

FIG. 18. — Transverse section of very young cat embryo: showing ectoderm, mesoderm, and endoderm. × 400.

endoderm. Where the animal is highly differentiated and a somewhat more complete early differentiation is needed, a third layer is formed between these two and is called the *mesoderm* (Fig. 18).

In such an early differentiation we have certain groups of tissues represented by the three layers, and to this extent the layers are apparently homologous in many groups of nearly related animals. But homology breaks down to such an extent when details are examined that it changes into an analogy when some larger groups are compared, and we come to see that the analogy is based upon differentiations that are responses to particular conditions under which any embryos, or the embryos of any group, must develop, rather than a fixed type of development which is similar because of blood relationship.

Protoplasm is slow to change its methods, however, and blood relationship, or common descent, probably does in a large degree determine many similarities of embryological development. The moment, however, that we attempt to compare the wider groups, we must recognize the possibility that even a large number of similar processes may proceed

from the methods used to meet similar conditions, and not from phylogenetic relationships, other than the common possession of a protoplasm which is subject to the same laws.

FIG. 19. —Segmenting ovum of *Loligo Pealii* to show early traces of bilateral symmetry. × 45. (After WATASE.)

The early bilateral symmetry of the young eggs of many animals is a preformation of the form of the body best adapted to the needs of motion (Fig. 19). Where there is no ancestral history of motion, or where this history is very far in the background, the animal develops with an upper and a lower pole and a number of radially symmetrical sides. The number of these sides varies from four sides in some medusæ to the greater numbers seen in the echinoderms or the theoretically infinite number in the sessile sponges.

When the animal is one that is to be moving in any part of its life history, the development tends toward the bilateral symmetry found in all animals that move forward. The bilateral symmetry may be superimposed upon the radial as is seen in the adults of some echinoderms, where radial symmetry extends into many planes, while that same radial structure is preceded by a bilateral form in the early embryo.

In the smaller and less highly organized forms the tendency is to form organs as soon as possible, that the creature may begin an independent life, securing its food and escaping its enemies by various motor and protective devices. These organs are often temporary, and replaced by other forms in the adult. The further details of the development of tissues will be considered, where they are necessary to a real understanding of histological structure, in connection with the descriptions of the various organs.

LITERATURE

Read the parts of Wilson, "The Cell," Schneider, "Lehrbuch der Histologie," and other general works that cover this subject.

CHAPTER V

MITOSIS

The growth of all tissues depends primarily upon the increase in the number of cells constituting the tissue. New cells arise only from parent cells by division of the parent cells. Since the life of any cell depends upon the presence of a nucleus, this process of division involves a dividing of the nucleus. In most cases of cell division the dividing nucleus elongates at right angles to the plane of cytoplasmic division. There are two types of nuclear division. The one is comparatively simple in the number of phases which it presents; the other involves a series of complex nuclear changes. The first is known as *amitosis* and is considered in another chapter; the second has been termed by various writers *mitosis*, *karyokinesis*, and *indirect division*.

The nucleus of a given species always undergoes in its mitosis a definite series of structural changes. The mitoses for various species present considerable variation. As mitosis has to do primarily and essentially with an equal division of the chromatin of a mother nucleus between two daughter nuclei, there is encountered less variation in the structural phases assumed by the chromatin than in the other structures concerned with mitosis.

The series of chromatin phases and their order of sequence is diagrammatically represented by Figure 20, *A* to *I*. The chromatin, which, in a resting nucleus, is more or less generally distributed within the nucleus as granules of chromatin (Fig. 20, *A*), is assembled to form in most cases a chromatin thread known as the *spireme* (Fig. 20, *B*). The nucleus now elongates, and in the higher forms the nuclear membrane at this stage disappears. The spireme thread breaks into a number of fragments. These may be rounded to rod-shaped bodies. Each chromatin segment is called a *chromosome*. In our diagram we have shown at *C* six chromosomes derived from the spireme. *It is highly probable that the number of chromosomes in a nucleus is constant for a given species.* For example, with the breaking of the spireme of a somatic nucleus from man, sixteen chromosomes will arise; the nucleus of *Ascaris megalocephala* var. bivalens represents four chromosomes. In certain mitoses the number of chromatin segments may vary from the specific number. It seems to be supported by evidence that when such variation occurs,

certain of the chromatin rods or all of them represent one or more chromosomes fused. These compound chromosome rods, when formed of two chromosomes, are said to be *bivalent;* where more than two chromosomes enter into their formation, they are said to be *plurivalent.*

The chromosomes formed from the segmenting of the spireme assemble in a plane, through which the cell will divide, forming what is termed the *equatorial plate.* In cases where the chromosomes are rod-shaped they usually become bent into V-shaped bodies. The apices of the bent chromosomes often converge so that the equatorial plate, when seen from above or below, presents a radiating figure [1] (*D*). In this position each of the mother chromosomes divide by splitting longi-

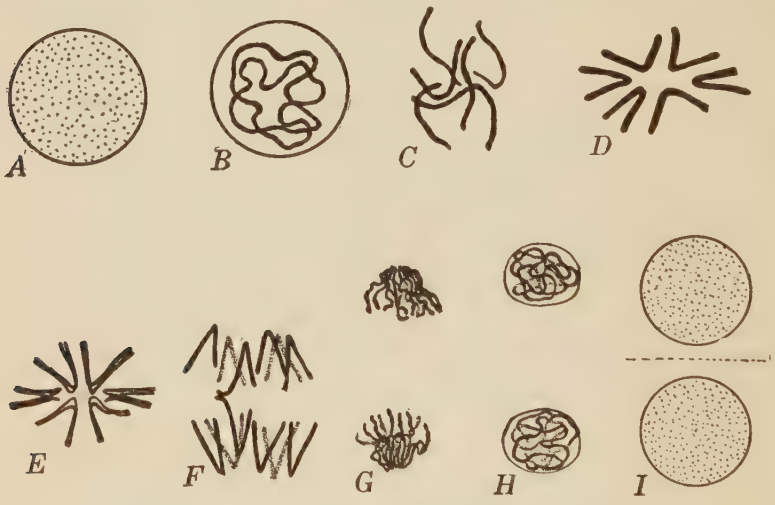

FIG. 20. — *A–I.* Diagrams of chromatin changes during the division of a cell.

tudinally into two daughter chromosomes. Two groups of daughter chromosomes are thus formed (*E*). One group is assembled above the plane of cytoplasmic division, the other below at right angles to it (*F*). Thus two groups of an equal number of chromosomes are brought to lie opposite each other with the plane of approaching cell division lying between them (*G*). The chromosomes of each group blend with one another to form a spireme. About each spireme a nuclear membrane forms, and we have the *dispireme* phase of the chromatin (*H*). The spiremes next become more slender and finally break up into chromatin particles to be distributed throughout the nuclear space (*I*).

As a result of this series of chromatin changes there are two groups of

[1] This is frequently spoken of as the monaster. The word *aster,* however, is given to another part of the mitotic figure; so for clearness of terms we do not here use the word *aster* for any figure formed by the chromatin.

chromatin in the same structural condition as was the chromatin of the resting mother nucleus (*I*). From the granular condition of the chromatin of a resting nucleus to the formation of the equatorial plate there is a progressive series of chromatin changes which carries the chromatin *toward* the plane of cell cleavage (*A* to *D* inclusive). The splitting of the chromosomes at this plane is an intermediate phase. Following this there is a regressive series of chromatin phases during which the divided chromatin travels away from the plane of cell division (*F–G–H–I*). The progressive phases we choose to call the *prophases;* the intermediate the *metaphase*, and the regressive series the *anaphases*. The chromatin during all these phases stains deeply; for which reason the figures presented by the chromatin in mitosis have been called the *chromatic figures of mitosis.*

In all mitoses there are other structures, much less constant in appearance in various species, which do not stain readily. These are called the *achromatic structures* or *figures of mitosis*. They furnish the path along which the series of chromatin changes are run, and perhaps they are the dynamic structures that control and direct the chromatin movements. The chief or most common of these is the *spindle* (see following numerous figures). Ordinarily the outline of this structure is fusiform. The shape, however, is not constant. It may be cylindrical- to barrel-shaped. In most cases the spindle is striated, showing that it is composed of delicate fibrils arranged longitudinally and converging at the poles. In a typical spindle there is a central bundle of fibrils, *the spindle fibrils*, extending from pole to pole, and a second set of fibrils which extend from chromosomes to poles. These surround the central fibrils and have received the name of *mantle fibrils* (Fig. 21). The spindle always lies at right angles to the plane along which the cell divides, with its equator in this plane, so that it is about the equator of the spindle that the *equatorial plate* of chromosomes is formed. As the daughter chromosomes separate, a plate of granules is formed, in many spindles, through the equator (see Fig. 34).

Fig. 21. — Mitotic figure, spermatagonium of *Podophyllum*. Shows the *spindle* fibrils reaching from pole to pole and the thicker *mantle* fibrils reaching from pole to ends of chromosomes.

These granules appear, one on each fibril, and they unite to form collectively a structure known as the cell-plate. The cell-plate spreads

with the advance of mitosis in such a manner that it may take part in the formation of the transverse cell-wall or cell-membrane.

FIG. 22. — Tripolar mitotic figure from nurse in pollen sac of *Magnolia*.

In most animals and in some plants the spindle of mitosis has at each pole an aster with its cytoplasmic rays, archoplasm and centriole. A spindle with its asters is termed an *amphiaster*. There is usually one spindle arising from a single nucleus. In certain plant tissues and in pathological tissues very complicated spindles arise with more than two poles. In the nurse cells of magnolia, for example, tripolar spindles are met with (Fig. 22). Figure 23 shows a multipolar spindle from a cancer tissue.

The spindle arises in many Protozoa and Protophyta within the nuclear membrane. Its origin within the nucleus has been described in certain plants such as *Erysiphe* and *Fucus* (Fig. 24). Until recently the aster was held to be as constant and permanent a feature of the cell as the chromatin. Recent evidence makes this quite unlikely. The aster with its rays, archoplasm and centriole, may as completely vanish as the spindle, to reappear when mitosis again ensues.

FIG. 23. — Cancer cell from man. Multiple division by many centers.

This complex nuclear activity is usually accompanied by a division of the cytoplasm of the cell. It occurs, however, in certain cells where there seems to be a demand for greater nuclear surface without cytoplasmic division. This results in multinuclear cells. We have examples of this in the giant marrow cells and in the male nurse cells of some plants. In the nurse cells of the anthers of *Magnolia*, for instance, there is an intermediate example, where we sometimes find mitosis with cytoplasmic division, and at other times without cytoplasmic division (see Fig. 383).

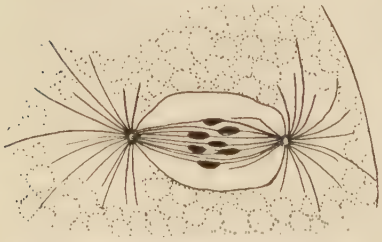

FIG. 24. — Mitotic figure in *Erysiphe*. Spindle formed inside of nuclear membrane (*n.m.*) After HARPER.

An Example of a Mitosis without Centrosomes. — For this mitosis let

us take that found in the root-tip cells of a hyacinth. The cells are six-sided, nearly cubical bodies, fitted to form concentric layers around a central row of slightly larger cells. The cells are of an average size for plant cells. They divide constantly in a plane usually at right angles to the length and direction of growth of the root. This division and the subsequent elongating of the newly formed cells results in the lengthening of the root. Examples taken about 10–45 cells back from the apex of the root will form the basis of our description and are represented by Figures 25 to 36.

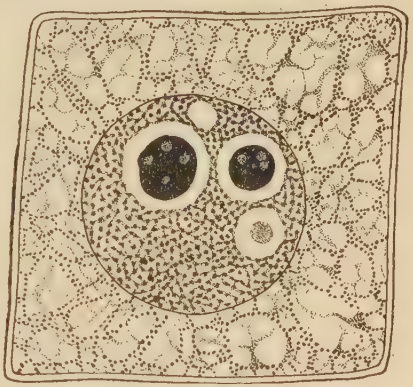

FIG. 25. — Resting cell in the root-tip of a hyacinth.

A cell while resting between divisions (Fig. 25) possesses a dense cytoplasm, of apparently alveolar formation, which at first sight is apt to give one the impression of a reticulum. Several vacuoles of large size are present in the cytoplasm, and these increase in size with the age of the cell. No especial organs are to be seen in the cytoplasm except an occasional vacuole containing spindle-like crystals. The nucleus is large, of a diameter over two thirds the average diameter of the cell, and round or slightly compressed in outline in the narrower cells. It is surrounded by a well-developed nuclear membrane.

The karyoplasm is dense and is composed of two visible substances, a deeply staining chromatin and a semitransparent linin. The chromatin is plainly seen to be divided into many small portions equally spaced through the karyoplasm and connected with one another by strands of the linin. Resting in the mesh-like karyoplasm are to be seen one or more nucleoli. If a single body, the nucleolus is found nearly in the center of the nucleus; if two or more exist in the same nucleus, they occupy the centers of portions of the karyoplasm proportionate in size to themselves. Each nucleolus always lies in the center of a vacuole. It always lies in the center of this space and never rests against the side, thus leaving a clear zone around its body and between its own substance and that of the chromatin-linin network. This indicates that the vacuole is occupied during life by a more or less solid substance of sufficient density to prevent the nucleolus from moving about. Besides the vacuoles which contain nucleoli, other spaces apparently without nucleoli are found; these are always smaller than the others and at first sight contain only the clear substance. A closer examination, however, reveals

central bodies in the larger of these spaces, and these bodies have a smoky and transparent appearance. The very smallest spaces contain nothing at all that can be seen with the microscope.

The nucleoli in nearly all cases contain vacuoles in their own substances, these vacuoles being filled with a substance which does not stain deeply, but to the same degree as the bodies in the smaller karyoplasmic spaces described above. These nucleolar vacuoles vary in size and number; they increase in both as the cell grows older. The clear fluid or substance which fills all the rest of the nucleus is a decidedly visible body in the cell we are studying. Although sufficiently clear and free from staining power to permit of our seeing all the other organs clearly, yet it is distinctly visible in cells fixed in Flemming's fluid. Its density or consistency is not known by any visible feature except perhaps the fact that by coagulation it supports some of the parts and organs of the nucleus. All the substances described are surrounded by the nuclear membrane, which forms a complete covering to the entire nucleus. This membrane has an appreciable thickness and is homogeneous in structure. It has considerable staining power, more than the linin and less than the nucleoli.

The first evidences of an approaching division are seen best in the chromatin. Its particles appear blacker and larger, and a close inspection shows them to be more irregular in outline and to have arranged themselves in incomplete rows in various parts of the karyoplasm, thus altering the granular structure to a thread-like appearance. A slight enlargement of the nuclei, the appearance of larger vacuoles and more of them in the nucleolus, which becomes irregular in outline, and the growth and complete alignment of the chromatin particles into threads now cause the stage to become easily identified and found among the cells (Fig. 26). The chromatin at this stage has formed a complete and apparently continuous thread, called the *spireme*, in the meshes of which the smaller and much distorted nucleolus is still seen. This thread is not only complete but its ends are joined, forming a Gordian knot which is usually broken or disturbed by the process of section cutting. It is of considerable length.

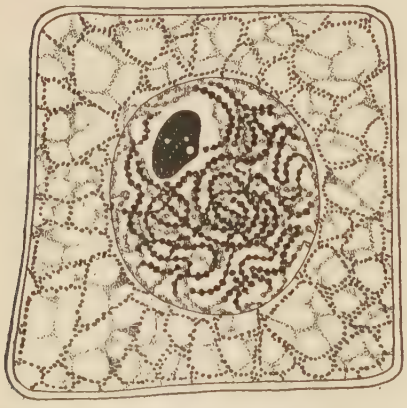

FIG. 26. — Hyacinth root-tip cell showing first signs of an approaching mitotic division. Chromatin gathering in skein or spireme.

Next to take place is a shortening of the spireme and a thickening of its diameter, accompanied by a merging or blending of the constituent chromatin granules into each other so that the spireme assumes a smooth, wire-like appearance (Fig. 27). Other phenomena soon take place. Two clusters of transparent filaments appear in the cytoplasm, one on either side of the nucleus and opposite each other.

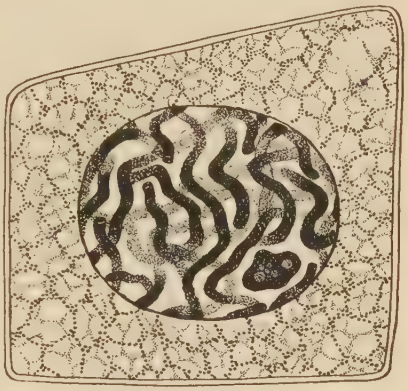

FIG. 27. — Hyacinth root-tip cell with spireme formed. Nucleolus dissolving.

Each cluster consists of many fibrils of an achromatic substance (Fig. 28). These fibrils are scattered evenly and closely on the nuclear membrane, about one fourth of whose surface they cover. They reach from there outward toward a common center in the cytoplasm, at which they converge. Thus each cluster of fibers forms a short, thick cone, placed opposite to its neighbor, and the two together form an incomplete diamond-shaped outline, which is called the *spindle* when a little further developed. Shortly after the spindle has begun to appear, the nuclear membrane begins to dissolve, becoming thinner, and finally disappearing at one or more points, but without losing at any time its rigid curved outline. It apparently dissolves *in situ*, and when once started, the breaches rapidly widen until all the membrane is gone. Its points of first disappearance are usually, but not necessarily, at the poles as indicated by the spindle.

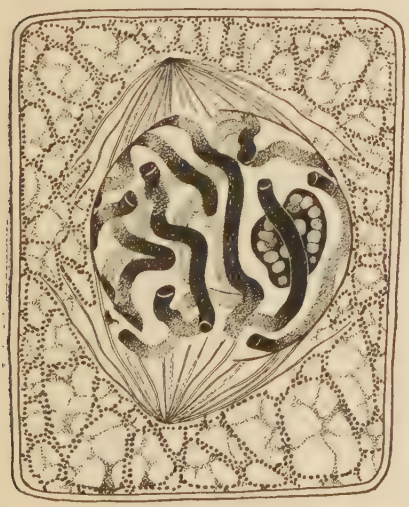

FIG. 28. — Hyacinth root-tip cell with spindle forming and nuclear membrane dissolved at two points. Nucleolus appears larger, but is smaller in bulk, and apparent size is due to vacuoles.

Shortly after the membrane has gone at these poles the spindle fibers grow into the nucleus and touch the chromatin spireme. The nucleus then develops into the somewhat more frequently seen stage pictured in Figure 29. Here three changes are apparent. The nuclear membrane is completely

gone, the nucleolus is gone, and the spireme is compressed into an oval to round mass, lying at right angles to the spindle and the plane of the equator, halfway from pole to pole.

FIG. 29. — Hyacinth root-tip cell with spireme entire but ready to break into the chromosomes. Spindle formed. Spireme appears to be divided owing to process of sectioning.

This round mass, which is a flat disk in the dividing cells of some other plants and animals, is still the continuous spireme noted before; in its later stages several breaks may be seen with the loose ends springing up from the main body. The spindle fibers, which are now developed into longer cones, touch the equatorial mass of chromatin and appear to have established a continuity through its meshes, so that a fiber starting at one pole runs without break through the spireme to the opposite pole. The breaks observed in the spireme (not shown in Fig. 29) increase until the whole spireme has been divided into a number of equal parts. There are twelve in the case of the hyacinth root-tip. These portions of the spireme, known as chromosomes, quickly move into one of the most commonly seen figures as pictured in Figure 30. Here the chromosomes are scattered as by the snap and momentum of their breaking, and yet two facts must be noticed; some part of each one is still retained in the equatorial plane of the spindle, and such parts of the chromosomes as have moved from this plane lie usually among and parallel to the fibers of the spindle. The wandering portion usually consists of a free end of a chromosome.

FIG. 30. — Hyacinth root-tip cell. Spireme newly broken into the chromosomes.

Ordinarily the wandering ends are found on each side of the equa-

torial region, but in many cases great indifference is shown in their distribution, and it may even be that all ends reach toward the same pole, leaving the other side empty of chromatin.

The change from the present stage as seen in Figure 30 to Figure 31 is marked by one very noticeable fact to which all else is subsidiary. This is that *every* chromosome as of one accord moves so that the exact middle of its length will lie directly in the equatorial plane. As an accompaniment to this, its ends are apt to straighten out in a variety of directions, so that a definite though irregular horseshoe-shaped loop, much elongated, is usually the result.

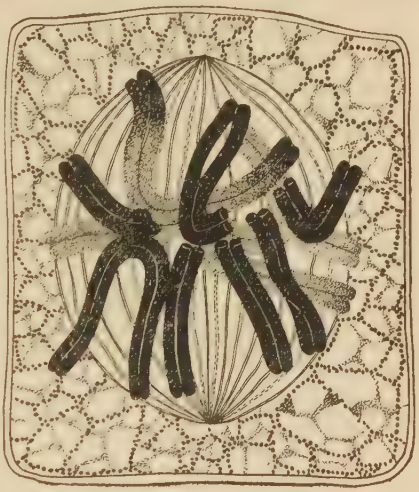

FIG. 31. — Hyacinth root-tip cell. Chromosomes doubled and with their points of first division in the equatorial plate.

While this is being done, another very important change takes place. Each chromosome splits for its whole length into two halves, the halves remaining attached for all their length by an achromatic substance which permits of their dual structure being seen. This stage figured in Figure 31 is rare. It is very quickly followed by the stage seen in Figure 32, where it is quite evident that each dual loop is being pulled apart by fibers attached to the apices, one fiber reaching from a pole to one half of each loop, while another fiber from the opposite pole is attached to the apex of each of the other daughter loops. Thus, when these fibers contract, the daughter chromosomes are well apart (Fig. 33). They move with ends straight out and form a common and characteristic figure.

FIG. 32. — Hyacinth root-tip cell. Division of the chromosomes.

The question naturally arises as to the motive power involved in this

transportation of chromatin. The chromosomes seem to possess the power of independent motion, as witnessed by their movements in Figures 30 and 31; when pulled apart as in 33. However, the very probable and entirely possible motive power is the contracting fibril. In Figure 33 the fibrils between these two masses of separating daughter chromosomes show us that not all the fibrils of the original spindle were employed to draw the parent chromosomes apart. Consequently we are able to distinguish between the two kinds, the remaining fibrils being called true spindle fibrils, and those used in the division mantle fibrils, because they are placed on the outer surface of the spindle.

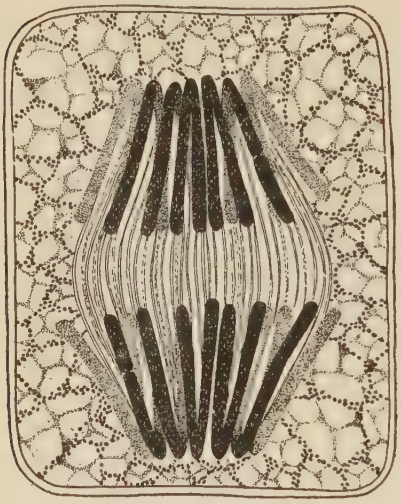

FIG. 33. — Hyacinth root-tip cell. Chromosomes reaching the spindle poles. Spindle fibrils shown between.

The spindle, whose form and poles were so perfectly seen in Figures 29, 30, 31, and 32, is now, for the first time, seen clearly at the equator from which the chromatin has moved. This leaves the equator free but obscures the poles, which will not be seen again because the chromatin is so thick and dark. The spindle as seen in this stage is composed of true spindle fibers only, as the mantle fibers were withdrawn while dragging the chromosomes to the poles.

Two changes mark the next stage which is represented in Figure 34. First, the chromosomes have shortened and thickened and become compacted into a denser mass than at any other time in the whole process of division. They have partly lost their individuality by blending together as though being of wax which had been subjected to heat. Secondly, there has

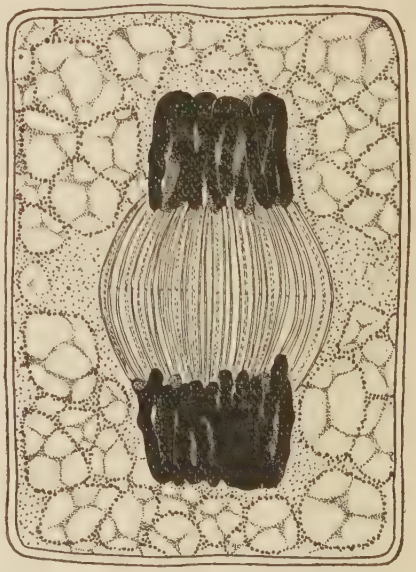

FIG. 34. — Hyacinth root-tip cell. Massing of the newly divided chromosomes. Beginning of a division in the cell-plate.

appeared in each spindle fiber at the equator of the spindle a small thickening. These lie in the same plane and form the cell-plate.

In Figure 35 one can easily see that the coalescing mass of chromosomes has become surrounded by a nuclear membrane of somewhat peculiar shape, while the chromatin itself has assumed a spireme-like shape which in proportion and texture much resembles that of Figure 27. The whole series of changes through which the chromatin goes in this part of the division process has been compared to a reversal of the earlier changes. It is so to a very limited degree and with regard to the chromatin only. Figure 35 also shows an increase in the row of dots which have become stronger and formed the cell-plate, which is the plane through which the two daughter cells are shortly to separate.

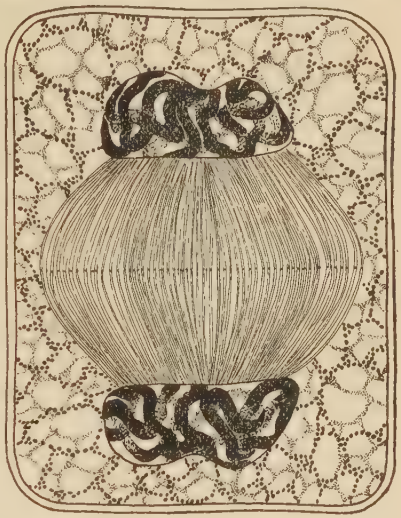

FIG 35. — Hyacinth root-tip cell. The nucleus divided and the daughter nuclei reforming. Widening and further cutting off of the cell-plate.

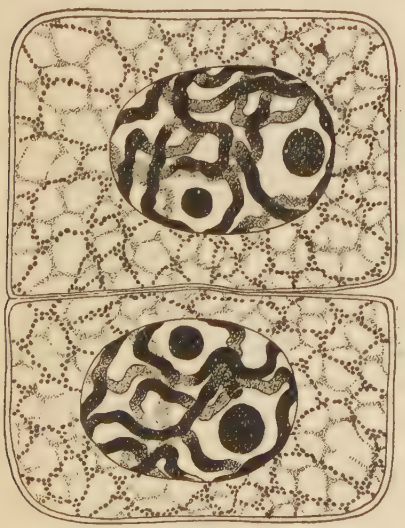

FIG. 36. — The two daughter cells of hyacinth root-tip. Reappearance of the nucleoli.

Our last Figure 36 shows the two daughter cells practically separate and complete; the nucleolus has suddenly reappeared; the chromatin is nearer to that seen in Figure 26 and can easily be traced to that stage or to the stage shown in Figure 25 or the resting cell. The nuclei are enlarged and rounded in outline and the achromatic fibrils have all disappeared. It but remains for the two daughter cells to grow in size and then to begin the division cycle over again.

An Example of Mitosis with Centrosomes. — We select the *ovum of Unio* for the demonstration of mitosis with a complete achromatic figure because of its availability. The ova are found from the early days of May to the late days of June in various stages of segmentation.

The first and second segmentation stages afford the best demonstration objects. The nucleus of these blastomeres is, in its resting stage, about fifty micra in diameter with an oval to rounded contour. The karyosomes and plasmosomes stain deeply and are variable in number; usually three or four are present. The largest one is generally a deeply staining body with a vacuole of lighter staining material through which are seen granular strands of the chromatic material (Fig. 37).

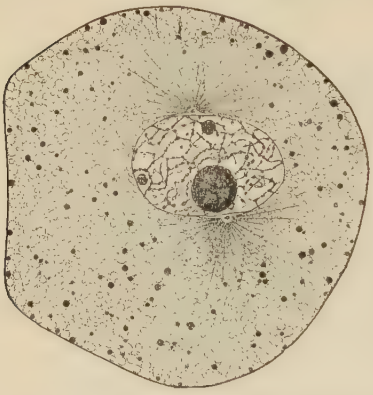

FIG. 37. — Beginning of fourth cleavage division of cösperm of *Unio*. Centrosomes appearing on sides of nucleus.

The chromatin is distributed as a series of fine particles throughout the nucleus and is supported by a reticulum of tough, non-staining linin which can be brought out and made visible by careful staining with eosin. As in many other resting cells, the strands of linin tend to start radially from the largest nucleolus and stretch irregularly to the periphery of the nucleus as a network. The nuclear membrane is sharp and well developed. It stains with most dyes.

The resting nucleus shows no trace of a centrosome inside of it or outside in the cytoplasm. The first sign of an approaching division, besides an increase in the staining power of the chromatin, is the sudden appearance of a centrosome on each side of the nucleus. These structures are in the cytoplasm, but rest closely against the nucleus. Each centrosome consists of a large centriole lying in a very small centrosphere from which long, delicate rays pass out into the cytoplasm.

At this time the linin disappears and the chromatin begins to gather into larger masses (Fig. 38). The nucleolus often, but not always, shows much vacuolation. The centrosomes grow in size and apparent strength.

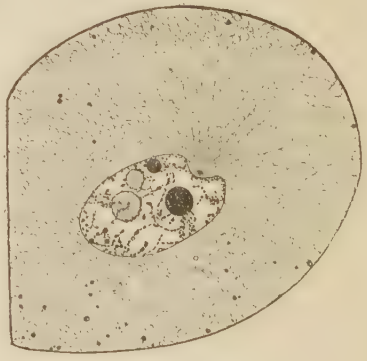

FIG. 38. — A slightly later stage than in last figure. Chromatin gathering. But one centrosome shown in this section.

Figure 39 shows the next important step. The chromatin has formed a very long and thin spireme, some parts of which are thicker than others. The nucleolus is very much smaller, and the centrosomes are beginning

to move apart. As they move they seem to leave a vacant, cone-shaped area between themselves and the nuclear membrane. The outer boundary of this space seems to be the straight rays from the centrosome, which are very well developed and are numerous enough to form a continuous boundary (Fig. 39). A new and weaker set of radiating fibrils appear in this space, and reach from near the centriole to the nuclear membrane, upon which they appear to have some destructive influence. This membrane begins to curl where so influenced, and in the next figure (40) it is shown as very much degenerated.

FIG. 39. — Later stage than Fig. 38. Irregular spireme, centrosomes moving apart and nuclear membrane beginning to fail where touched by the forming spindle fibrils.

This figure also shows the chromatin spireme broken up into a certain number of long, bent chromosomes, which are not yet arranged in the equatorial position which they must occupy before they can be drawn apart

FIG. 40. — Later stage of this mitosis. Nuclear membrane nearly gone. Spireme breaking to form chromosomes. Spindle fibrils growing towards each other. Nucleolus almost gone.

in longitudinal halves. The spindle fibrils which were so weakly developed in Figure 39 are here seen to be more strongly developed than

the astral fibrils. These latter are very long and stretch out into the cytoplasm almost to the cell-wall in some places. The chromosomes are mossy at this time and the nucleolus sometimes persists as it has done in our example.

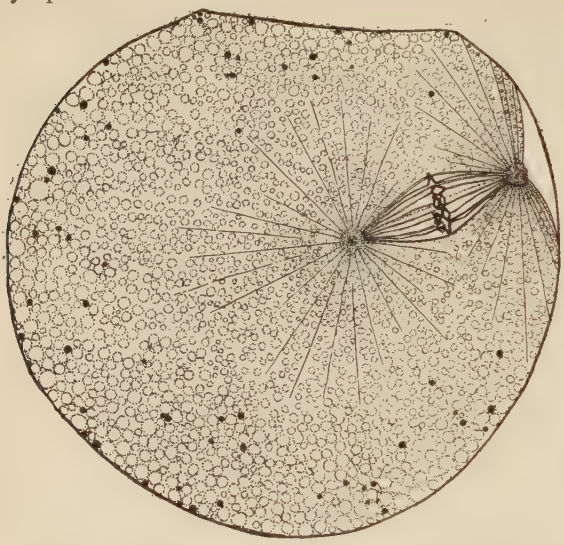

FIG. 41. — Same process at time of formation of equatorial plate of chromosomes.

Figure 41 shows the figure completed and ready for the division of the chromatin. The spindle fibrils are at their best development, and some of them can plainly be seen to have become attached to opposite sides of the chromosomes and be pulling them apart. The chromosomes are shorter and smoother than they were in the preceding stage. They are bent or V-shaped rods which are first split at their apex. It can be seen in this figure that the strain on the spindle has caused a sinking in of the whole surface at the point where this strain is greatest.

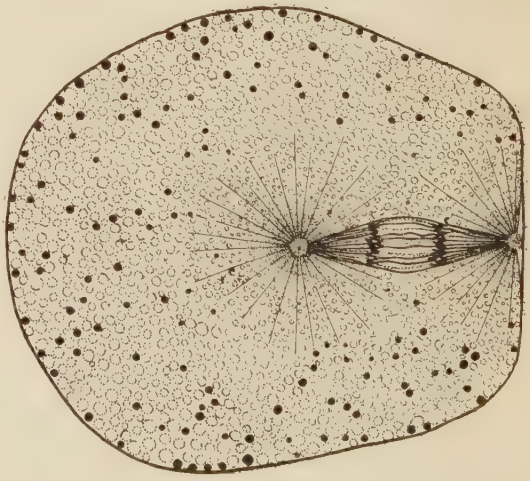

FIG. 42. — A cell of the same kind showing separation of the chromosomes.

A peripheral layer of the cell is left in its original position. The next figure (42) represents a stage, subsequent to the last, in which the chromosomes have been drawn apart. The form is well shown. As in the hyacinth figure, the spindle fibrils are shown between the parting groups of chromosomes, while the fibrils which are seen between the chromosome groups and the centrosomes

must represent both kinds of spindle fibrils, the rigid and contractile fibrils of the achromatic division figure.

Figure 43 shows the reforming daughter nuclei. The vestiges of the spindle and the process of division of the cytoplasmic body are both well shown. The nucleolus is slow to reappear and all traces of the centrosome have disappeared.

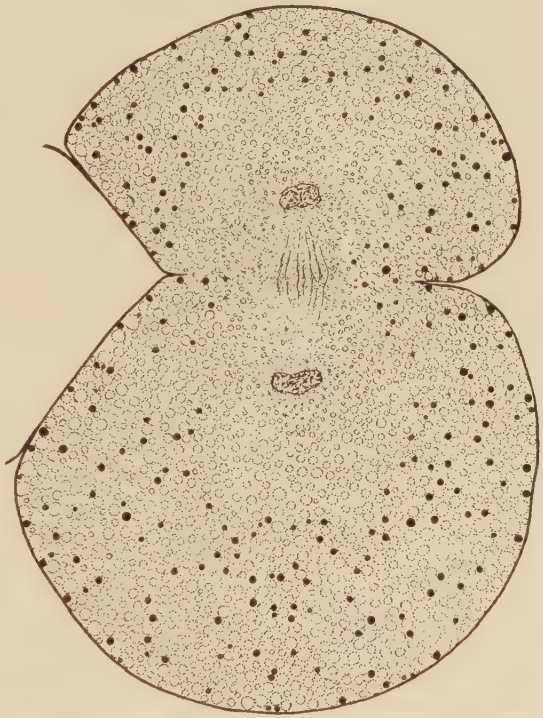

FIG. 43. — Division of nucleus almost completed. Cell beginning to divide.

Technic.—Flemming's fluid and sublimate are recommended for this work, together with the paraffin sectioning method and iron hæmatoxylin staining. There are no special methods other than a few of the analine dyes to make differential stains of the various parts of the dividing cell. Great care is necessary not to injure the delicate structures by any rough usage. Too quick dehydration will sometimes injure the specimen.

LITERATURE

The same general works should be read as were recommended for the last chapters.

LILLIE, F. R. "Organization of the Egg of Unio," etc., *Journ. Morph.*, Vol. xvii.

AMITOSIS

Another kind of cell division is found in which the complex processes studied in mitosis are not present, and the cell divides by a series of autoconstrictions of first the nucleolus, then the nucleus, and lastly the cytoplasmic body. This is known as *direct* or *amitotic* division. Strangely enough this method corresponds almost exactly with Remak's description of cell division when he first "discovered" and figured it upon very

slender observation in the chick. His observations, however, were made on tissues that divided by mitosis, and he probably mistook some later phases of this process for a process superficially comparable to amitosis.

Amitosis usually begins in a cell by the elongation of the nucleolus at right angles to the future plane of division of the cell. A constriction of the middle of this organ then proceeds, not as though some power was cutting it in two as a band or string would do, but more as though the two ends were being pulled apart and the middle was thinning out to the breaking point. The two daughter nucleoli then move apart to positions in the approximate centers of the two future daughter cells, and the nucleus is ready to divide. Frequently the daughter nucleoli begin to elongate as though for another division before the mother nucleus has even begun to divide.

In other cases the nucleus acquires the two daughter nucleoli not by the splitting of the old one but by the growth of a new one *in situ* at the opposite side of the nucleus from the original nucleolus. This method often results in a splitting of the nucleus before the new nucleolus is as large as the other, and one of the new cells is then smaller than its sister-cell.

The division of the nucleus, subsequent to that of the nucleolus, may be done in one of three ways, the first and second of which are much alike. It may divide by a constriction of its body, as in the case of the nucleolus: this, however, is rare. Oftener it forms a plate at the plane of division, and this becoming double, the two plates separate, showing flat and parallel surfaces where the separation took place. The third method of nuclear division, recently suggested by Child, 1907, from numerous observations on many forms, is performed by the formation of two new nuclear membranes inside the old one and around the daughter nuclei. This is strongly suggested by his figures, one of which is copied in our Figure 44. The dissolution or absorption of the old membrane would then leave the two daughter nuclei free in the cytoplasm and separate from one another.

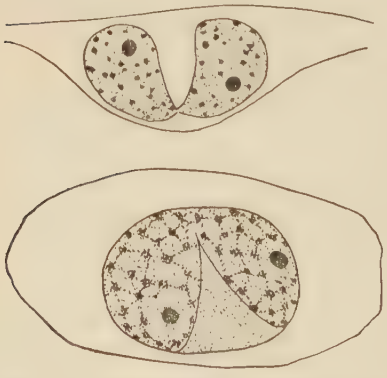

FIG. 44. — Amitotic division in the tissues of a flat worm, *Planaria maculata*. (After CHILD.)

The cell body is last to divide in amitosis, and in many, perhaps the majority of cases, it does not do so at all. Or it may form a division

boundary like the cell-plate, and then never finish by a complete separation. Most frequently the cell forms from two to several nuclei and goes no farther, dying when its duties are finished, which may be a short time in certain follicle cells and stratified epithelia, or may be as long as the life of the animal, as in the case of the muscle cells.

The commonly received idea, at present, concerning amitosis is that it is a terminal process in the cell's life activities and is a method for securing more nuclear surface for use in forced metabolism or secretion. We have three well-defined cases to examine, out of the many that could be mentioned, to prove that this is highly probable.

First, the follicle cells of the cricket's ovum show an easily read history that can be interpreted in no other way (Fig. 45). The ovary of this insect, like that of many others, is composed of a number of chains of about seven distinguishable ova each. In each chain these are continually being added at the top in a small and immature state, while at the same time they are being discharged from the lower end into the reproductive passages in a large and mature condition. The ovum grows many times in bulk and surface area during its descent, the greatest increase occurring in the lower end of the chain. Each ovum is covered with a single layer of flat epithelial cells, the follicle cells, that have the work to perform of transforming the food materials, brought by the blood, into yolk material and passing it on to the ovum, which is storing it up for future use. This layer of cells is fastened to the ovum and accompanies it from the beginning to its discharge.

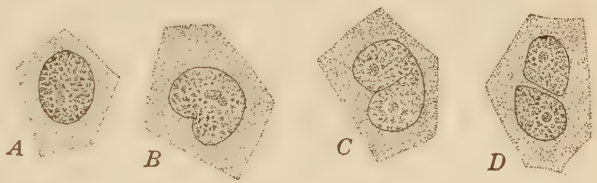

FIG. 45. — Four stages in the mitotic division of a cricket's egg-follicle.

The follicle cells increase in size during the descent of the ovum, but not at an equal ratio to the increase of the ovum's surface. Therefore they increase in both numbers and in size to keep the follicular covering complete. The increase in numbers occurs only in the upper end of the chain, and is done by mitotic divisions of the cells. When the ovum is in the lower portion of the chain, these same cells divide by amitosis, which is somewhat incomplete because only the nucleolus and the nucleus divide, the cell body remaining intact in all cases observed. The follicle cells increase in size at this time, however, to keep the follicle large enough to cover the growing ovum.

Amitosis is clearly not a means of cell multiplication in this tissue. Since it occurs, not only in this well-defined case of rapid metabolism,

but also in many others, we have some right to connect it with these processes and to infer that its principal object is to provide increased nuclear surface as quickly and with the least expenditure of energy possible.

A second example is described on page 90 (see Fig. 87). Here, again, the nucleus divides by mitosis, while the cells are increasing in number in the primitive muscle regions. When the cells are sufficient in number and begin to form fibrillæ, the nuclei rapidly multiply by amitosis, probably to afford more nuclear surface to the growing cell, which is now secreting the heat- and motion-producing substance in increasing quantities.

The third case that we shall examine is a very common one, and is found in nearly all stratified epithelia, especially in the higher vertebrates (Fig. 46). Here, as in the follicle cells of insects, the nucleus divides by mitosis to increase the number of cells; but it changes to amitosis, without a division of the cell body in the latter part of the cell's life. Again, the probable object is to enlarge the nuclear surface for increased metabolism, the formation of keratin in this case. Strangely enough the specialized parts of this epithelium that produce certain oils and scents show no signs of amitosis in their later stages of secretion and degeneration (see Chapter XX).

FIG. 46. — Amitotic division in the middle stratum of stratified epithelium from the roof of a Guinea pig's mouth.

The above cases could be multiplied indefinitely. As was seen in the preceding part, mitosis is also used in them for other purposes than growth; but we are now confronted with the question, can amitosis be used for any real growth purposes?

Opposed to the preceding general ideas as to the meaning of amitosis we find the conclusions of Child (1907) and others who, in very recent papers, apparently show that amitosis occurs very extensively as a factor in the growth and regeneration of many young and unspecialized tissues, including even the reproductive tissues. This is described in tissues from most of the principal groups of animals.

The observations on which these descriptions are based are as yet "somewhat fragmentary" according to the chief observer, Child (1907). And yet we must recognize that if they contain any truth at all they must contain a great mass of facts that will seriously conflict with the prevailing ideas. Meves' observation on the reproductive cells of the

salamander seem to support and afford a firm foundation for Child's work.

We have, then, apparently, another kind of amitosis which cannot be explained in the same way as the follicle cell's divisions and other terminal kinds can. It appears to be the same process morphologically, but produced by different conditions and leading to different results that we cannot, as yet, differentiate from the conditions that control the first kinds of amitosis mentioned.

Technic. — The technical methods for the last two sections are the same as for the section on the cell. The cricket's egg follicle epithelium is best studied by fixing the ovaries in picro-acetic, washing in alcohol, and then stripping the epithelium off with needles and staining it with a weak solution of methel green and acid of fuchsin. Great care is necessary to avoid taking the tough egg membrane off with the epithelium, as it takes the methel green so strongly that nothing else can be seen through it.

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CHAPTER VI

EPITHELIUM

EPITHELIUM is a tissue whose cells line all outer and inner surfaces of the animal body. In this position these cells are called upon to make all transfers of material from the outer world into the tissues of the body and from the body to the exterior. Epithelium must also act as an agent for the transfer of sensation and for the mechanical protection of the organs from the rubs and knocks of the surrounding media and objects.

Its position, with its cells touching the surface of the body, is its chief distinction, and it is only in consequence of this position and the duties that accompany it that the cells are modified into the great variety of structure that we find among them individually and collectively.

These cells have a strong polarity, a differentiation into an inner and an outer surface or end. These ends differ strongly, according to the work that they have to do, the outer end usually being more highly differentiated than the inner. This difference accords with, first, the physiological fact that the outer ends of the cells are subject to the greater variety of conditions which determine their differentiation; and secondly, materials for the elaboration of excretion and secretion products are taken from the intercellular fluid or blood by the inner ends of the cells. These substances are all much alike. By a series of processes this material is finally delivered at the outer ends of the cells in the form of excretion or secretion particles. The variety of these elaborated bodies gives a greater variation to the outer than to the inner ends of the epithelial cells.

The sides are arranged, with rare exceptions, for but one purpose, that of fitting together with those of the surrounding epithelial cells. The commonest shape of cell, in consequence, is that with six sides, approximately conforming to the mathematical requirements of the occasion. The number of sides is variable in different epithelia or even in the same one, and owing to irregularities of arrangement may be more or less than six. Sometimes there are many sides in some cell that is placed in a position where it is surrounded by a large number of others.

The cells of an epithelium are cemented together by the sides more or less firmly, but at the point or line where they touch each other, at or near

the surface, there is developed a peculiar "closing plate" or *terminal bar* that serves to close the whole layer of cells into an impervious layer or covering (Fig. 47). The terminal bar is probably impervious to all gases, fluids, and other materials, and it remains for the cell to determine what shall and what shall not pass into or out of the body. This bar is double in section, as can be seen when the two parts are separated by dissolving the cement substance that holds them together (see Fig. 47). Seen from a surface view, the bar is rod-shaped, but it is not altogether a continuous structure, being rather a series of closely set granules. These granules are specializations of the *desmochondria* found on the surface of most cells, and the cytoplasmic fibrils that end in the desmochondria are also found ending in the closing plates. These same desmochondria are found all over the surface of the stratified epithelial cells of the mammals. Here the individual desmochondria that compose the bars are entirely separate and give the surface of the cell the appearance that was formerly known as the prickles on the prickle-cell. The desmochondria that compose the terminal bars of most cells of assimilation are particularly easy to see individually. In all simple epithelial cells the terminal bars, since they lie between all cells, are united into a reticulum, the meshes of which conform to the outlines of the cells. This appears in a surface view of the epithelium.

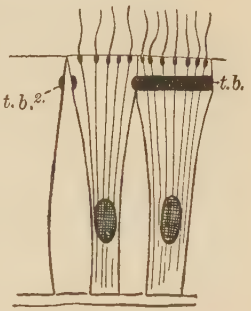

FIG. 47. — Diagrammatic figure of two cells and the side of a third. *t.b.*, terminal bar seen from a lateral view on the uncut side of a cell; *t.b.*², cross section of another terminal bar whose two parts are somewhat separated.

We must keep in mind that these various features of the epithelial cell, polarity, shape together with the large differences in structure due to the differences in function are, as is mentioned above, the result of their position on an inner or outer surface of the body. This idea can be excellently understood by studying the dividing oösperm of the frog or other amphibian, which will clearly show the origin of epithelium and some first causes of its differentiation from the other cells of the body.

The oösperm is at first a single cell whose entire surface touches the exterior (Fig. 48, *A*). There are no inner surfaces at this time. A division into two, then into four, and again into eight cells, makes every cell in the developing body have an outer surface and three sides, but no inner surface, as the three sides bring it to a point. All cells are to be considered as epithelial cells at this time, although they perform all the functions of the body (Fig. 48, *B*).

The next few divisions of these cells result in the formation of some

cells that do not, in any way, touch the outer surface. These inner cells have been differentiated apart from the epithelial cells which now lie between them and the outer world, and where the two touch is found a surface that is the inner end of the epithelial cell (Fig. 48, *C*). Repeated divisions now reduce the size of the cell in proportion to the size of the organism until we find the condition seen in Figure 48, *D*, where the epithelial cells form a flat, even sheet of tissue covering every part of the body. Many other cells have divided off from the outer or epithelial cells and have been added to the inner mass of cells. Some few epithelial cells (*i.e.* cells touching the outer surface) have been crowded out of the outer row and pushed into the inner mass, where they can be distinguished by the pigment lying in what was formerly their

FIG. 48. — Four stages in the cleavage of the ovum of a toad, *Hyla pickeringii*.

outer end. Other inner cells have been crowded by the inner growing mass out into the epithelium, a rarer case than the former. Out of all this change only such cells as maintain a position on the outer surface become the epithelial cells. Their use is determined by their position, not by any structure or predetermined feature that we are able to demonstrate. After the period of differentiation, when the epithelial cells have attained their final characteristics, they can no longer take up the position or function of a mesoderm cell; nor can the latter become an epithelial cell in the higher forms. In the lower forms these exchanges of position or function are often possible and take place during regeneration.

Each epithelial cell has a flat, even, outer end that unites with the ends of its surrounding neighbors in conforming to the surface of the body. The inner ends of the cells, at first very irregular (Fig. 48, *C*), soon become

aligned to form an inner surface (Fig. 48, *D*) which later is provided with a complete membrane that lies between it and the other body-cells lying inside it. This is called the basement membrane, and differs much in the amount of its development. It is not always present, and may be thick and irregular or thin, tough, and smooth, and the cells sometimes project through it into the tissue beneath. It provides openings for nerves that come from the interior to end in the epithelium (see Fig. 192), and for lymph channels, or even for blood vessels, when it is otherwise so dense that the lymph could not pass through.

The relations of the cells to the basement membrane are not always the same. The simplest formation is with all the cells in a row and their proximal ends resting squarely on the membrane. Or they may only touch the membrane with one or more processes (see Fig. 192). Lastly, some of them may grow up and away from the membrane altogether, being supported by contact with those that remain in the original position. This is true of the perceptory cells of the auditory or static epithelium of vertebrates (see Fig. 192) as well as of some other forms.

This last principle, when carried farther, results in many cells arising from the row of the simple form of epithelium and lying in outer positions. They remain attached to the inner cells that are on or connected with the basement membrane. Such is called a stratified or multiple epithelium, and is met with in the integument of most vertebrates and some invertebrate animals (Fig. 49).

FIG. 49. – Epithelium from the head of *Sagitta hexaptera* to show a simple stratified epithelium. (From SCHNEIDER.)

In the skin of embryonic mammals can be seen splendid series of developmental stages of this form of covering. Here the epithelium starts as a simple columnar form, a plain row of cells in section; in reality a single sheet of six-sided cells covering every part of the embryonic body. On the umbilical cord this epidermis begins to change into a stratified epithelium at a number of distinct points, one of which is shown in the illustration (Fig. 50). It can be seen that the cells that form the outer portions are not actually lifted out of the basal layer and pushed outward, but that the cells of the basal layer divide by mitotic division, and that the outer cells resulting from such divisions lie outside of the basal layer.

They afterward divide one or more times by themselves, this time by an amitotic division, and are then pushed farther outward by the next divisions of the cells in the basal layer. The amitotic divisions do not appear in such early stages.

Thus, the basal layer remains where it started, continuing to divide mitotically, and only such of its daughter cells as can keep in contact with the basement membrane remaining basal cells. All others, having left the basal layer, divide once or twice more by an amitotic division and are pushed continually outward. When a certain distance from the basal layer, they fail to get the proper amount of nourishment, and die. The outer layer of dead cells is, as can be seen, constantly accumulating and must be as constantly reduced in order to keep its volume at some normal point. This reduction is performed by the removal from the outer

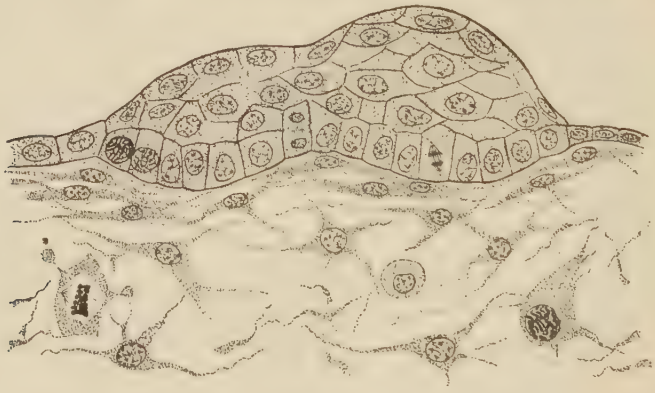

FIG. 50. — Section of a single point of stratification on the umbilical cord of an embryo sheep. Mitosis in the basal layer and in the underlying connective tissue.

surface of as many of the dead cells as is necessary, by abrasion, by the shedding of layers of these cells, by the solution and decay of some of them on moist surfaces, and in certain cases by the processes of oil formation that result in their degeneration and destruction when the product is set free. They are also used by being built up into various defensive and offensive structures, as hairs, horns, feathers, etc. (see Chapter XX).

An epithelium formed in the above manner from a simple or single layered covering of cells is known as a *stratified epithelium*. Besides being derived from the simple form of epithelium, it usually shows a vestigial arrangement of its basal layer in a columnar or simple form. As a secondary differentiation we may find the outer layer of cells elongated and placed in a row so that they look like the columnar form. Such cells are secreting cells, and **a good example may be found in certain folds of the conjunctiva of the young alligator,** where these cells are engaged in

secreting an oily substance for lubrication (Fig. 51). This last modification is rare, as can be imagined when we consider that all the materials to be used in secretion by the outer cells must be passed out to them by the epithelial cells that lie between them and the basement membrane. This calls into play an extra, and what nature seems to consider a needless, effort, and one that is avoided wherever it can be.

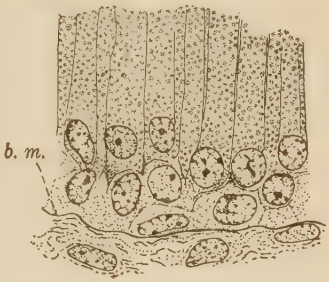

FIG. 51. — Pseudostratified secreting epithelium from the conjunctiva of the alligator. *b.m.*, basement membrane.

For such reasons the secretion of materials is nearly always confined to a simple epithelium, which, to get the required body of cytoplasm, is composed of much elongated cells, and thus becomes columnar. The secretion may be delivered in the form of a fluid or of granules. It may change from one to the other after delivery, and before its use, or it may be transformed into gas at or about the time of delivery.

The secretion is produced, sometimes at the expense of the cell, as has been mentioned in the case of stratified epithelium. In mucous and other columnar cells it is produced at the expense of the cell's distal cytoplasm, which is later regenerated.

The outer edges of simple epithelial cells show many modifications, intended to be of service to them in their activities. In other forms the production and delivery is steady and constant, and the cell is always in the same condition physiologically and structurally.

FIG. 52. — Cells from the digestive tract of a plecypod mollusk, *Cyclas*.

The cell-product is sometimes a hardened edge or a continuous cuticle which is produced jointly by all the surface cells. In other cases it is a striated border, which may or may not be furnished with cilia. **Cilia are shown in figure 52**, which represents the digestive epithelium of a small plecypod mollusk, *Cyclas*. As can be seen here, the cilia enter the cell and converge in a course through the cytoplasm until they arrive, as a single fiber, at the side of the nucleus.

Technic. — The very simplest methods are of the widest use in studying this tissue. Two special methods should be mentioned. The use of a little nitrate of silver by brushing it on the fresh tissue after washing with

distilled water will usually bring out, after exposure to sunlight, the outlines of the cells where they come in contact one with another. This is of great advantage in studying some kinds whose outlines are indistinct under other circumstances. The second method is that of *teasing*. The intercellular cement substances are easily dissolved out of most epithelia by the use of certain weak fixatives, as 33 per cent alcohol, very weak osmic acid, and weak chromic acid (see LEE). The individual cells can then be separated by gentle manipulation and examined by themselves. They may be stained and permanently mounted. A very useful but somewhat difficult method was used by the writers to study the relations of certain epithelia without entirely separating the cells. The nasal epithelium was somewhat softened and macerated in a number of media and then treated with one per cent osmic acid to harden and preserve it. It was then stained in several stains and carefully imbedded in paraffin; sections were cut and mounted without being affixed to the slide. The dissolving of the paraffin and addition of the balsam separated the cells enough to allow one to easily see them separately without having their relations to one another seriously disturbed.

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THE AMPLIFICATION OF BODY SURFACE

It is through the epithelial cells lining a body that its various material relations with the world are established, especially the taking in and the giving off of various substances. Several facts must be considered in studying these substance exchanges. One is that only a certain amount of transfer per unit of time can take place through a given unit of surface. And in an organism of high specialization this limitation is increased by the fact that there are many more different kinds of exchange to be performed than in lower forms; and usually each kind of work must have its own surface of a particular character.

The first attempts to meet these conditions consist of various gross arrangements of the body surface which are purely morphological in character and serve to considerably enlarge the square surface of the body. When these are completed, however, there is still not enough surface for the body functions of larger and more specialized animals to be carried on. Some way is necessary whereby this surface can be made to do more work without further increasing the surface morphologically.

What does occur to bring this about is, in reality, an increase in

surface. But it is an histological process that does not increase the morphological or primary surface, and all that it needs is a little greater thickness of this primary surface.

There are three stages of this process, resulting in three conditions. They are *corrugation*, *evagination*, and *invagination*, using these terms in a special and histological sense.

The first of these, *corrugation*, is the simplest, and consists of the throwing of an epithelial surface into a series of parallel folds on the connective tissue base (Fig. 53). This folding is not necessarily visible to the naked eye, and it may or may not yield and become flat when the surface is stretched.

Good examples of a real corrugation are somewhat rare in the adult organism owing to the fact that this process is usually but the beginning of the other two.

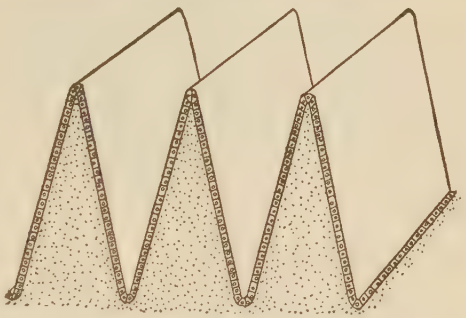

FIG. 53.—Diagram of a corrugated epithelial surface.

The corrugated side of the foot in some lamellibranch mollusks shows a splendid example of corrugation, with the limitation that it is sometimes partly obliterated by stretching, when the foot is extended. Sections at right angles to these corrugations are diagrammatically shown in Figure 53. It will be noticed here that one cannot say whether folds have been thrown up from, or depressions have been made in, the surface. **A second form of corrugation can be seen in the embryonic stages of the small intestine of vertebrates.** This stage is transitory, however, and soon passes into the next form of amplification.

This next form of amplification will be either that of *evagination* or *invagination*, and is determined by the way in which a cross folding is brought about. Figure 53 shows a simple corrugation, and we must now imagine that this folding has not sufficiently increased the surface and that a second corrugation is to be superimposed upon the first to increase its surface, not directly, for it can be seen in the figures that the surface is not mathematically increased, but by putting it in a form in which the specialization can be carried much farther than a plain corrugation could be. A cross corrugated surface would not be torn so easily, and the lumen would allow fluids to pass more easily through it than if it were lined with deep folds.

This second form of amplification is produced by making a series of depressions at right angles to the original folds (Fig. 54). These may be said to determine the original folds as depressions rather than ridges.

The result, then, is a surface marked into a series of elevations by two series of grooves at right angles to each other, and with the areas thus marked off rising into a set of elevations. These elevations are known as *evaginations*, and they may be very long and close set or fewer in number. They are not always formed by two successive groovings of the surface, but may arise from it simply as outgrowths.

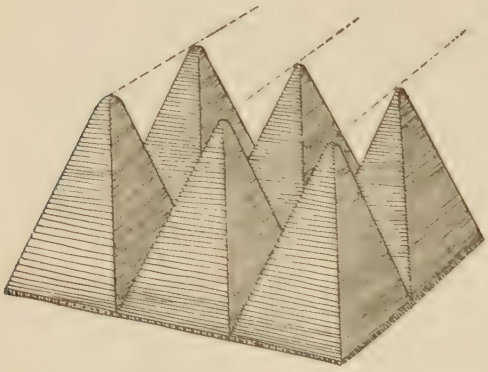

FIG. 54. — Diagram of an evaginated epithelial surface.

In the case of the example that we shall study, **the villi of the small intestine in some vertebrates**, the process of formation of the evaginations is approximally the same as the purely theoretical discussion outlined above has indicated, the first amplification of the surface of the lower small intestine in man being in the shape of grooves that are roughly parallel and not straight, but arranged in a sinous course. The division of the ridges lying between these grooves into papillæ by a second set of grooves at an angle to the first begins at an early date, in fact, before the first grooves are fully formed. In the upper large intestine of man this process can be best studied owing to the fewness of the evaginations, but after the villi are formed, they pass away and are not seen in the adult organ.

This furnishes an ontogenetic case of a process that may also be seen in a taxonomic series. The early taxonomic example is the adult intestine in many fishes, in which the amplification has only proceeded as far as a grooving.

On the whole this process of amplification by *evagination* is a rare one. The mechanical and physiological advantage seems to be all in favor of the third, which is that of *invagination*.

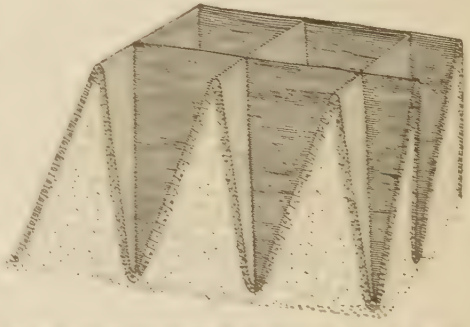

FIG. 55. — Diagram of an invaginated epithelial surface.

This process consists (theoretically and many times actually) of the *raising* of a series of ridges across the surface of the original folds, at

an angle to these folds, which are thereby determined as ridges (Fig. 55). This calls our attention to the fact that the folds in Figure 53 represent either ridges, or grooves, or both, as the case may be determined by other circumstances.

The placing of these two folds at an angle to each other leaves a series of pockets between them which are called *invaginations*. As both sets of folds are formed at the same time, we are not accustomed to think of them as such, but to direct our attention to the points which appear to be, and in most cases are, depressions from the surface. If they do originate between the folds, they subsequently are extended by a real inward growth or invagination.

A good example of such a structure may be studied in the stomach of a common carp. In a fish of moderate or small size the origin of these invaginations can be traced to longitudinal *corrugations* of the surface, as seen lower down in the intestine, and thus we can realize the relations of these surfaces. An examination with a strong lens of these various surfaces on freshly killed material, washed and treated with some hardening reagent, should follow or accompany the microscopic work.

The last specimen to be studied is the duodenum of some small mammal that has been carefully hardened, and bulk-stained, and cut. This should be studied in sections cut in the vertical and horizontal planes as well as one cut in an oblique plane.

We understand that a section of an organ is only an image of it in one plane. For this reason a perfect vertical section of either a corrugation, or an evagination, or an invagination, cannot be distinguished from a similar section of the other two. We may also see how a section of any one of these will reveal its true nature by many slight departures from a typical form. Also how it may deceive one by sections taken in the valleys or through the ridges of one or the other of them.

In considering any amplification of an epithelial surface in this work it will be designated as an evagination or an invagination, according to the relations which it bears to its free or distal surface. When the fold moves proximally, and contains a morphological lumen derived from the free surface, it will be called an *invagination*. When, on the other hand, it moves upward, inclosing a core of the tissues on which it rested, it will be termed an *evagination*. Thus the optic cup with its stalk will be considered as an invagination, although many embryologists speak of it as an evagination.

Technic. — No special methods for this part.

LITERATURE

Same as for the last part.

GLAND FORMATION

Glands may be defined as portions of epithelial surface used for the secretion or excretion of some particular substance or substances from the body. This surface may be small, in fact it may consist of a single cell, and often does. It usually consists, however, of quite a number of cells, and there may be several different kinds of cells among them.

This surface may be a part of some general body surface, but most often it is removed from the surface to which it belongs by an invagination. It may be corrugated or evaginated, but the commonest form is an invaginated gland.

We shall study as an example of a unicellular gland the mucous cell found in the digestive epithelium of the worm *Cerebratulus* (Fig. 56).

FIG. 56. — Portion of digestive epithelium of *Cerebratulus lactatus*, showing a deep mucous cell. *n*, nucleus of mucous cell. × 1200.

This epithelium shows, in section, a straight row of cells that are used, evidently, for digestive purposes. At intervals among this layer of even cells appears one which is so large that its body has grown down out of the row and become many times its original bulk. It is forced to do this by the kind of work that it must perform, the secretion of mucus. This necessitates the large bulk of cell body, and as the surface would lose its value as a digestive (and motor) surface were these great cells to spread the others apart and wedge themselves in between, the large mass of the mucous cell is kept below and only its distal end, with the average diameter of the undifferentiated cells, remains in the row. The nucleus is placed in the bottom of such a cell. See also the mucous cell of *Helix*, pictured by Figure 338.

An example of a unicellular gland that does not descend into the tissue below the epithelium of which it is a part can be seen in the goblet cell of the digestive epithelium in the intestine of any vertebrate animal (see Fig. 263).

Multicellular glands that have not been invaginated are rare. Where the unicellular mucous glands are collected closely on a surface, as in some mollusks, and also where primary surfaces of the digestive tract are used to produce some special fluid, we have examples of such *surface glands*.

By far the greater number of multicellular glands are evaginated from the primary surface. The simplest way in which this can occur is as a mere single pocket which may be a simple tube-like depression called the *tubular*, or a bag-like enlargement called the *alveolar* type of gland.

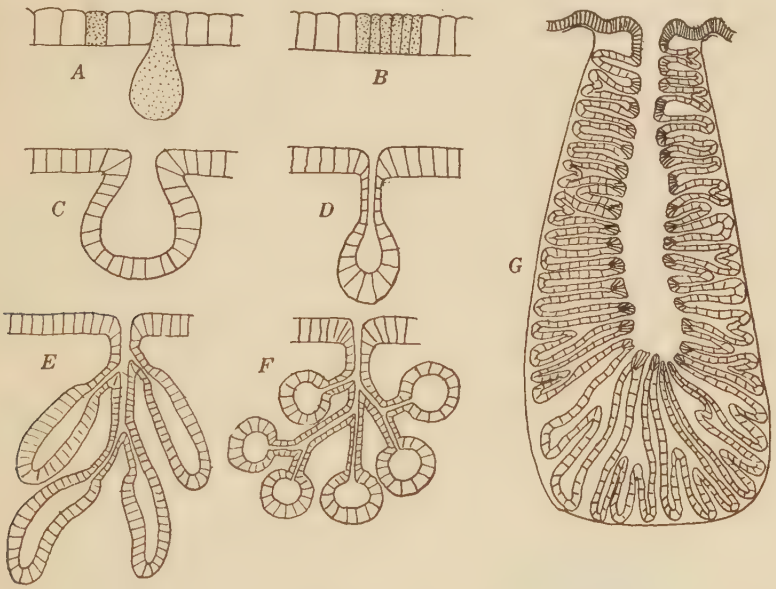

FIG. 57. — *A–G*. Diagram of the different sorts of glands. *A*, unicellular glands lying in the epithelium and one of them projecting out of it; *B*, multicellular gland not invaginated; *C*, simple saccular gland; *D*, simple saccular gland with neck elongated into duct; *E*, compound tubular gland with individual ducts collecting into a single opening; *F*, compound saccular gland; *G*, highly compound gland with one primary and many secondary invaginations. Only the secondary epithelium secretes.

These simple glands are the kind that usually produce the amplification of a body surface (Fig. 57). Sometimes the lower part, or fundus, of this simple gland does all the active secretion, and the upper part forms the tube or *duct* that carries the secretion from the fundus to the surface. When the fundus becomes a highly differentiated, saccular region on the end of a duct, it is called an *acinus*.

Many glands are compound in that they consist of two or more acini on the ends of a branching duct, which acts as a common carrier for their secretions. Compound tubular glands are to be seen in the digestive gland of the lobster and the pepsin glands of the mammalian stomach.

Compound saccular glands appear in the salivary glands of the mammals, of which easily studied examples are to be seen in the lower part of the tongue.

Besides such compounding of the simple glands a second sort of amplification may appear in which the walls of a primary acinus may be invaginated into acini of a different character. These we shall designate as the *complex glands*. **A particularly good example of such a complex gland** is to be seen in the stomach region or proventriculus of birds, of which the pigeon will furnish us with a good example (Fig. 57, *G*). In this specimen a section will show that each of the numerous glands opening into the lumen of the proventriculus is a simple saccular form lined with an epithelium that is different from that of the surface upon which it opens. The exact use of this primary gland epithelium is not known, but judging from its homologies it should be pepsin producing. Its staining reactions and general appearance, as well as the fact that the chief ferment of digestion is produced elsewhere, would indicate that this function has been lost. Opening from all sides into this primary fundus are many smaller, secondary invaginations. These are lined with a totally different kind of cell whose function has been found to be that of producing hydrochloric acid. These cells are represented in Figure 268, *B*, in Chapter XV.

Another complex gland is also a digestive gland whose primary and secondary invaginations produce digestive ferment and hydrochloric acid respectively. This is the tubular (usually) gland found in the stomach, and we shall examine the **form seen in the muskrat** (see Fig. 268, *A*). The point to be made here is that in the mammal acid gland we have a complex gland whose secondary elements are *unicellular* glands that have been slightly retired from the primary glands as secondary invaginations.

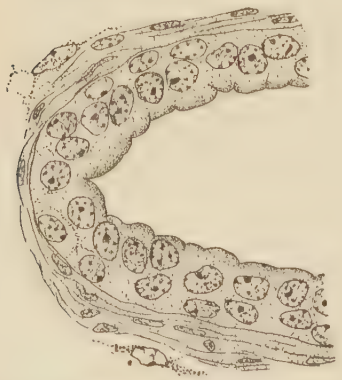

FIG. 58. — Transverse section of part of a medium-sized duct of the cat's submaxillary gland. × 870.

The ducts of glands form an interesting study. The duct is usually an intercellular passage with from three to six cells forming its walls at any one transection. On the one hand it may become much larger when it carries off the secretion products of very many gland bodies. In this case it is furnished with strong connective tissue coverings or even muscle layers to strengthen and contract it (see Fig. 58). On the other hand, the

gland duct may be reduced in size until it is an opening between two contiguous cells, as is to be seen in the mammalian liver. Or it may be a passage through the embracing arms of a single cell. This is well shown in the earthworm's nephridium (see Chapter XIX).

Lastly, the terminal proximal branches of a duct enter often into the body of the cytoplasm to form the intracellular gland ducts.

CHAPTER VII

THE SUPPORTING AND CONNECTIVE TISSUES

Protoplasm as such has not sufficient tensile strength or rigidity to enable the multicellular body to preserve its proper form. A certain type of cells is found which are devoted to this function. They fulfill it by the formation of *fibers*, *plates*, and *masses* of material, either on the surface of their cytoplasm or within it. These extra-cellular fibers, plates, and masses are made sufficiently strong, rigid, or elastic to meet the requirements for supporting that portion of the body in which they are placed. They are controlled absolutely in their development, growth, and change by the cells from whose cytoplasm they originate. We may call them the cell organs of support or, collectively with the cells that made them, the *connective tissues.*

The resistance afforded by the connective tissues to the body is of two kinds: A *binding* or *connecting* power secured by thin, strong fibrils in larger or smaller bundles, and a resistance to *impact*, *shearing*, or *bending pressures* met by rigid masses, shells, or rods. We can distinguish in consequence two classes of these tissues: The *tensile* or binding and the *rigid* or supporting connective tissues. It can be seen that all rigid connective tissues have some tensile strength and *vice versa*, but yet the predominant character of the tissue can be easily determined. In some tissues the two kinds are mixed to meet peculiar conditions.

Of each of these tissues there are two kinds. In one case the cell organ of support is an intra-cellular organ and is formed inside the cell. This more primitive method is rare and only occurs among the lower forms of life. In the second case the cell-organ is formed outside of the cell, and the same organ (fibril, plate, or mass) is usually formed by several cells jointly.

Among the vertebrate animals the chemical constitution of the extra-cellular connective material is used to classify the tissues into several groups. As this classification breaks down when extended into the invertebrate phyla, we shall not use it. For this classification the reader is referred to some good medical histology.

Adaptability and extreme range of variation are characteristics of the connective tissues, which develop or change to meet all kinds of requirements in the growth, renewal, and regeneration of the organism. These

changes of the tissue are produced by an increased formation, or a destruction of, the connective materials under the influence of the cells to which they belong. No tissue responds more quickly than connective tissue does to a sudden development due to exercise. When the muscles enlarge and grow stronger by practice, the tendon and the bone both do the same at the same time and at the same proportional rate of speed.

Technic. — This will be indicated under the following parts.

LITERATURE

Read general works, especially the early discussion of connective tissues in Schneider.

THE SUPPORTING AND CONNECTIVE TISSUES: SIMPLE RIGID FORMS

A Primitive Form of Rigid Connective-tissue Cell. — "Leidig's cell of the first order" in a crustacean, *Homarus*. In various parts of the internal anatomy of the lobster and nearly related Crustacea are placed masses of supporting tissue. These masses uphold and support the various delicate tissues about them and protect them from the impact of surrounding organs. The largest and most regular of the cells which compose this tissue are known as "LEIDIG'S cells of the first order," named after their discoverer (Fig. 59).

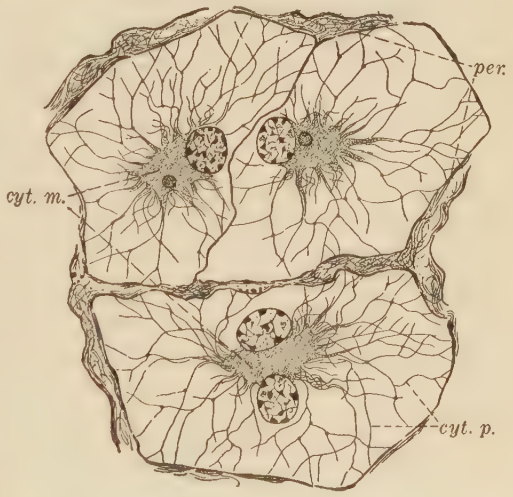

FIG. 59. Connective tissue cells from the lobster. *cyt.m.*, cytoplasmic mass; *cyt.p.*, cytoplasmic processes; *per.*, peripheral layer of cytoplasm on which the rigid material is laid down.

The large, well-formed nucleus occupies the central part of each cell. It appears to lie outside of the main central body of the cytoplasm. This is due to its extreme eccentric position in the cytoplasm, the bulk of which forms a dull gray mass lying apparently alongside of the nucleus. This cytoplasmic mass shows in its body a darkly staining area that is round in outline and much smaller than the nucleus. This is probably the centrosphere, containing the centrosome.

The important feature of this cell is the distribution of its peripheral cytoplasm, which is drawn out into fine-branching processes that radiate from the central mass. The processes arrive at a common boundary which lies concentrically about and at some distance from the main cytoplasmic mass, making this its center in preference to the nucleus. The processes expand their ends on this boundary, joining with the expanded ends of the other processes. The outer cytoplasmic boundary, so formed, is a very thin shell or hollow sphere sometimes a little irregular in shape, and it is about this shell of cytoplasm and by its agency that the rigid supporting material of the tissue is formed. The amount and arrangement of this substance, which is homogeneous and darkly staining, is determined by the mechanical requirements to be met at this point. Figure 59 shows a group of three of these cells where the supporting substance is not as great in amount or as specialized in arrangement as in the second and third orders of LEIDIG'S cells.

The peculiar arrangement of the cytoplasm, in threads radiating from the central mass and ending in the peripheral boundary, results in a cell which is not filled by its cytoplasm. This is explained when one realizes what a large and unwieldy cell would result if the cytoplasm should fill the cell entirely. The amount of cytoplasm would be excessive, and life could not be supported in the tissue. This peculiar distribution of a smaller amount fulfills all requirements.

A body of such cells is a tissue admirably adapted to its use. The compact mass of such rigid shells forms a framework which possesses sufficient rigidity to protect the delicate organs which it surrounds from impact and shearing strains imposed by the heavier surrounding organs.

The renewal of this tissue is easily seen, especially in the lobsters that are about to cast off their old shell or have just done so, as in the specimen used in this demonstration. The nucleus divides by amitotic division and the two daughter nuclei move to opposite sides of the cytoplasmic body. A line of separation then appears, passing through the cytoplasmic body and extending from periphery to periphery of the cell. The resulting daughter masses of cytoplasm then move apart. They carry their nuclei on the side farthest from the line of division. The specific connective substance of the cell is then laid down in the dividing septum, which thus becomes a new side to each of the daughter cells (see Fig. 59). It is of interest to note that, during multiplication, these cells lose no part of their functional power, but continue to function as connective-tissue elements.

We shall next, in pursuance of this idea, study the cells in the **growing root-tip of a plant, selecting that of the chestnut** for the purpose.

In plants, nearly every cell in the organism is, in addition to what other features it may possess, a rigid connective-tissue cell. This is almost

axiomatic and can be easily demonstrated. The development of the cell and its rigid connective-tissue organ is shown to great advantage in a longitudinal section of a growing root-tip. We have selected a root-tip from the chestnut seedling. Young cells selected from near the tip are solid masses of cytoplasm containing a nucleus (Fig. 60, *A*). They are surrounded by a well-defined cell-wall of some strength and thickness. This wall is the connective-tissue material and is visible from the very first. When a little further developed, a vacuole appears in the cytoplasm, and later one or more others appear near the first. As the cell grows larger, these increase in size and push the nucleus over to one side. In an older cell the two or more vacuoles have broken through the intervening walls of cytoplasm and united to form one large vacuole (Fig. 60, *B*).

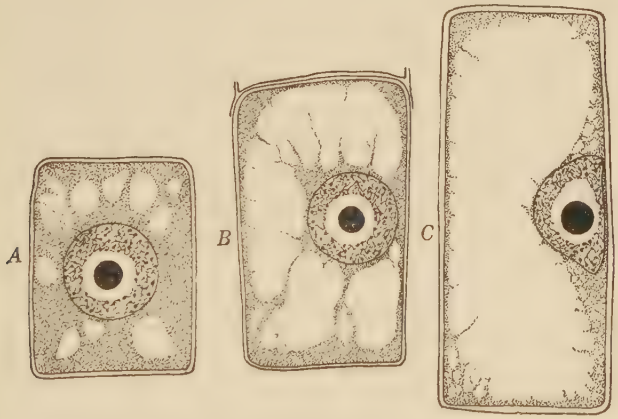

FIG. 60. — *A–C*. Three stages in the development of a plant cell as an organ of rigidity.

In some cases more than one vacuole is left. At this time it will be seen that the size of the cell is very much greater, especially in length.

The vacuoles increase in length until we find a mere shell of protoplasm lining the thick cell-wall and a larger mass of protoplasm somewhere nearer the center (Fig. 60, *C*). This mass contains the nucleus and is connected with the peripheral shell by strands and bridges of cytoplasm. During life, a circulation of the whole mass keeps the nucleus in touch with, and in command of, its most distant portions of cytoplasm. Thus the peripheral shell is able to make and preserve intact the important cell-wall, which is the cell-organ in this case and provides rigidity. The cell has enlarged during this development.

The same result, a relatively greater surface on a given mass of cytoplasm, is thus attained as it was attained in the Leidig's cell of the lobster. On the other hand, one should notice that there is no renewal, that the cell once formed goes through its direct form of development and

then becomes fixed in its permanent form. This permanent form varies in different plants, its highly developed form being the wood-cell, which is a cell in which the cytoplasm has developed the cell-wall out of cellulose and then has added another and more efficient material, xylem, that, in the aggregate, forms the ordinary wood of the larger plants and trees (Fig. 61).

FIG. 61. — *A–C*. Three kinds of wood fibers. (From STRASSBURGER after SCHENCK and SCHIMPER.)

One other example shows us a simple rigid connective-tissue cell that is constructed to perform its functions in a slightly different way. **The notochord of the vertebrates** is an organ that is typically an organ of rigidity (Fig. 62). It is composed of cells that during their development form a single large vacuole in their center to secure a large surface on a small body of cytoplasm. The outer portion of this cytoplasm secretes a shell of dense, firm material, whose strength, in connection with that of all the other cells around it, gives to the notochord its characteristic firmness. In the process of vacuolization the cytoplasm is pushed from the center to the periphery, where it forms a shell that secretes the cell-wall. This cell-wall is the cell-organ of rigidity. The cytoplasm inside of this connective-tissue shell is much thicker at one point than anywhere else, and the nucleus is located in this thickened mass. This form is a temporary embryonic one and has no renewal process.

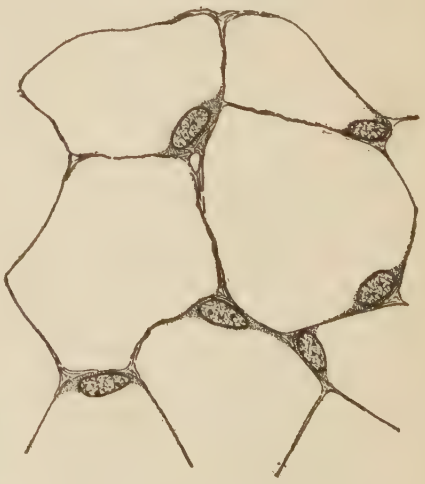

FIG. 62. — Notochordal cells from an embryo toad fish, *Opsanus*.

It should be noticed in all the above examples that the vacuoles, which play such an important part, are filled with a fluid which, for a better knowledge of its use and constitution, will be called the *cell-sap*.

We now turn our attention to what is an extremely primitive but comparatively rare form of rigid connective tissue: This is **the spicule-forming cell of certain sponges** (Fig. 63). Many cells of the mesoglœa of such a sponge form in their cytoplasm a tiny pointed rod of calcium

carbonate. This grows until it gets too large for the cell and its end sticks out.

At this time it is used for a rigid support for the body of the sponge in connection with many other similar rods. The spicules (Fig. 63, *A*, *B*), as these structures are called, assume a great variety of forms. (For the supporting cells in certain integuments, see Chapter XX on the "Integument.")

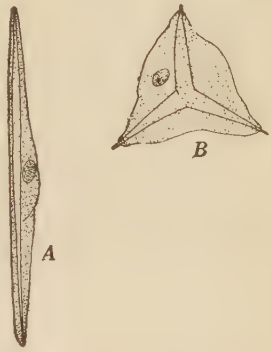

FIG. 63. — Developing spicules of a sponge. (From SCHNEIDER after A. MAAS.)

Technic. — In general, fixatives containing acids, particularly acetic acid, should be avoided in the preparation of connective tissues. The rigid forms are least damaged, especially when they are chitinous in nature. Flemming's fluid and sublimate should both be used for such examples as are used in this part. Sublimate is neutral in its action, and is very useful where it is desirable to study the cell structure as well as a spicule or other extra-cellular formation. This sublimate must always be carefully removed by means of iodide of potassium (see chapter on technic).

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THE SUPPORTING AND CONNECTIVE TISSUES: PRIMITIVE TENSILE FORMS

Examples of cells that bind the parts of a body together are universal. The simplest form is seen to greatest advantage in the embryos of all animals before the extra-cellular substance has assumed such proportions as to obscure the cell and its nucleus. An example is found in the **umbilical cord of a sheep** about one quarter advanced in its intra-uterine development (Fig. 64).

The tissue lying directly under the external epithelium is composed of a number of cells spaced at somewhat regular distances from each other. Each cell has its nucleus placed in the middle of the cell body. This

nucleus is unspecialized and similar to the other tissue nuclei in its neighborhood.

The characteristic feature is the cytoplasm. This is drawn out into tapering strands, of which two to four or five are usually present in the plane of section. These arms of cytoplasm meet the arms from other cells and form all together a network which fills the space and holds everything together by the union of the arm-like processes (Fig. 64).

As before stated, the cytoplasm is not in itself strong enough to do the work that these tissues will be called upon to do. The required strength is furnished by bundles of fine thread-like bodies of great strength, which appear about the cytoplasmic processes. These threads are formed by the cytoplasm and controlled by it. They extend from

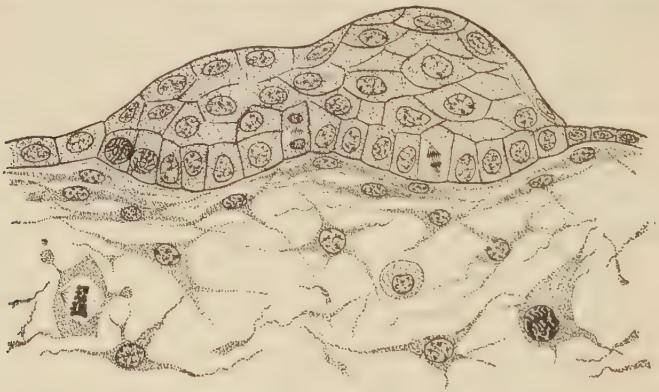

FIG. 64. — Part of a section across the umbilical cord of an embryo sheep. Under the epithelium are seen the young connective-tissue cells with branching cell bodies that form a reticulum. These cells divide by mitosis, two stages of which may be seen in the figure.

cell to cell and by becoming united to similar neighboring bundles of threads at various angles, they form a very strong network of tissue. They are not strongly formed at this time in the specimen.

The extreme adaptability of such a tissue forms an interesting study in early embryonic sections. The cells which ordinarily reach and hold in all directions are, when placed in some special position, rapidly modified to meet any unusual strain from any given direction. Thus, close to the epidermis, they flatten out in a plane parallel to the surface to protect the parts from injury when stretched or punched. Around the blood vessels they elongate into two-armed spindles, which encircle the tube and hold it while the blood comes in waves and spurts against its walls.

The growth of this tissue is performed by mitotic division, as shown in two stages in Figure 64. When fully developed, most of it forms the so-called lax connective tissue between muscle and skin and the fascia of muscles.

Such tissue can also be found in many very low animals and the embryos of most invertebrates. In vertebrate embryos, besides being under the epidermis of the umbilical cord, it is found under the skin of the whole body and among all the organs.

Neuroglia, because of the form of its cells, might be placed in this class of connective tissues. Because, however, of its association and common origin with nervous tissues, we have placed it with the nervous tissues in Chapter XIII.

Technic. — Carefully made sections fixed in most of the ordinary fixatives will give good pictures of the cell elements. When it is desired to see the extra-cellular fibrils, a fixative without acid must be selected. Both paraffin and celloidin sections should be used. The stain can be a special stain for the demonstration of the fibrils, as may be seen by going over the stains for this purpose in LEE. Teasing has been of no use for the study of these tissues.

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THE SUPPORTING AND CONNECTIVE TISSUES: SPECIALIZED TENSILE FORMS

It is right to decide the degree of specialization of a tissue by the degree to which its characteristic features are developed either quantitatively or qualitatively. Thus we may examine, as a specimen of highly specialized tensile connective tissue, **the tendon connecting a muscle with a bone in a mammal or bird** (Fig. 65).

The first impression one receives on looking at a longitudinal section of tendon is a great amount of fine, strong, parallel strands in which no nucleus is present. Nuclei can be seen, however, between the fibrous strands; and a closer inspection, especially in transverse sections, will disclose the cytoplasm belonging to each nucleus, wedged in between the fibrils and, like them, drawn out extensively in the direction of the fibrils. The entire surface of the cytoplasm is in contact with some fibrils. In turn each fibril throughout its length is in contact with the cytoplasm of some cell.

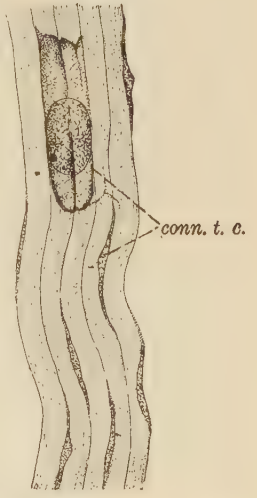

FIG. 65. — Portion of tendon from a cow. *conn.t.c.*, connective-tissue cells seen from the side and, in one case, from the surface.

It will now be realized that the fibrils are not cellular elements, but extra-cellular structures belonging to and elaborated by the cells we have found lying among them. The cells present several odd and extreme features by which they differ from a typical cell. The nucleus is not spherical, but drawn out so that it appears in our specimen like a bit of red thread and needs a high power to show its nuclear organs. Again, the cytoplasm does not lie as a spherical body around the nucleus, but is drawn out from it in three, four, or five longitudinal and lateral plate-like projections, which have been appropriately called "wings," leaving a very small proportion of the cytoplasm immediately surrounding the nucleus. It also reaches for a considerable distance beyond the two ends of the nucleus.

The nucleus is long and thin because it must lie in the midst of fibrils which, at frequent intervals, are under enormous strain. If spherical, the nucleus would be crushed and would interfere with the efficiency of the fibrils. The cytoplasm is drawn out in several directions because it must be in contact with the fibrils to support them. It is drawn out in the wing-like form because this form allows its duties to be performed without injury to itself or interference with the work of the fibrils. It is a manifest mechanical truth that a given number of fibrils all pulling together should be placed parallel to each other and side by side for the greatest efficiency, and the call for efficiency is so urgent in the highly organized tendon that the cellular elements have to be modified to give the fibrils every opportunity.

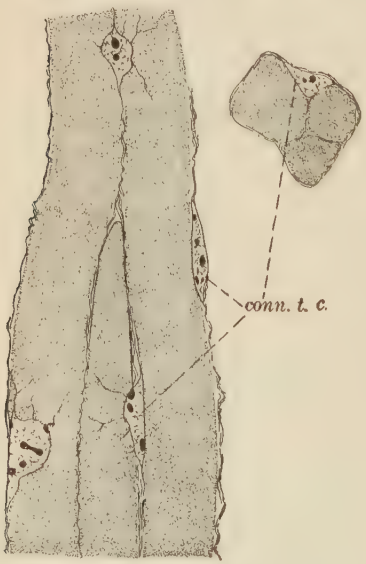

FIG. 66. — Portion of ligamentum nuchæ of ox. *conn.t.c.*, connective-tissue cells.

The tendon we have examined is not elastic to any degree, but will break before it will stretch. This condition is doubtless obtained by its chemical composition and demanded by its use. There are other connective-tissue cells, however, which form tendons that will stretch.

The ligamentum nuchæ of an ox or other mammal shows this structure (Fig. 66). Here the cells have a different structure that produces an elastic fibril in place of the non-elastic fibril. This elasticity is further developed by the network-like arrangements of the fibrils which are woven among each other and do not lie parallel. The fibrils are very large and the nuclei of the cells

are flattened out somewhat, but not drawn out as in the tendon. These cells seem to have formed a second set of extremely minute fibrils which lie between the large, heavy fibrils of elastic tissue and at points appear to be attached to them. These loose fibrils appear to be a lax connective tissue.

The lower forms of animals show but few examples of specialized ligaments, owing to the general distribution of the muscles and their large areas of attachment. Also many of these forms have no skeleton, and consequently the muscles work in masses of connective tissue and in a series of opposed planes.

The Crustacea have a rigid exoskeleton or shell and a series of powerful, quick-acting muscles that require firm attachment to the inside of the shell. They are attached by very short ligaments, as is described below. No long ligaments exist, and therefore when a muscle does not reach, by some little distance, to its point of attachment, an inwardly produced process of that point, composed of hypodermis and cuticle, reaches in to meet the muscle. These integumental "ligaments" are characteristic of the Crustacea and may be seen by examining the muscle of a lobster's great claw.

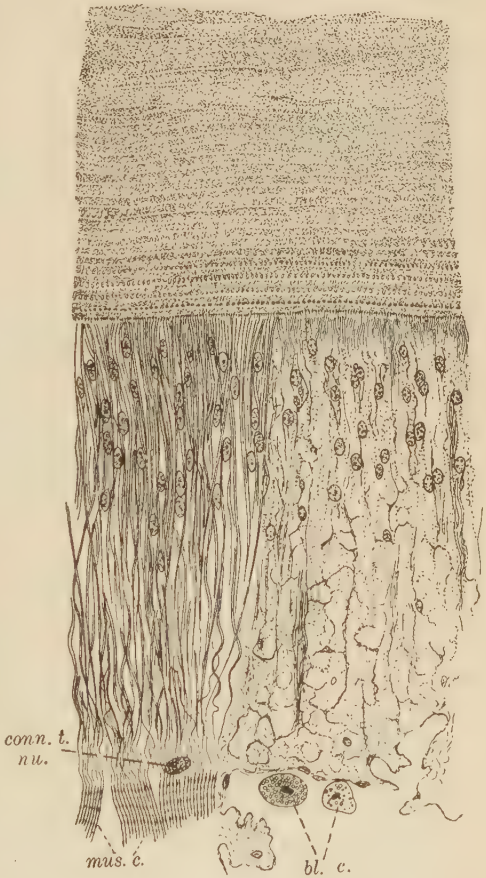

FIG. 67. — Portion of the new integument of a lobster, *Homarus*. *conn.t.n.*, connective tissue nucleus; *bl.c.*, blood cells; *mus.c.*, muscle cells.

A tissue, however, that acts as a very short ligament does exist in the lobster wherever a muscle is attached to the shell or to one of its inside processes. It is not connective tissue alone, however, that serves this purpose. The simple, columnar epithelium cells that cover the outside of the body under the shell, which they form, assume part of this duty and acquire strength to

perform it by the development of strong fibrils in their cytoplasm.

Figure 67 shows a bit of shell resting on the columnar epithelium that formed it. In the right-hand portion of the figure is seen this epithelium in an undifferentiated state, with its base resting on a delicate layer of connective tissue. The exact boundary between the base of the epithelium and this connective tissue is difficult to detect. In the left-hand part of the figure can be seen the same structures, but somewhat modified, owing to the fact that some muscle fibers are attached to them at this point.

Both the connective-tissue cells and the epithelial cells have modified their structure and united to act as a short ligament. The fibrils which were weakly developed in the undifferentiated epithelial cell have become heavy and strong, and lie in parallel positions in this differentiated one. The weak fibrils that run in irregular courses and partly parallel to the shell in the first set of connective-tissue cells have become stronger and assumed a position of greatest efficiency, at right angles to the shell and in line with both muscle and epithelial fibrils, in the tissue seen in the second group. As in most connective-tissue cells, the actual boundaries are too complicated and weakly marked to be seen.

These three sets of fibrils, the epithelial cell fibrils, the connective-tissue cell fibrils and the muscle fibrils, have been joined individually into a set of long and apparently continuous fibrils, of which the first two portions form the passive ligamentous part, while the last acts as the actively contractile portion. The strain thus comes, in all three of these cells, not on the cytoplasm of the cells but on special cell-organs which have been formed by the cytoplasm to meet it.

This mode of muscle attachment is found throughout the lobster's body. In some places the connective-tissue elements are so small as to be apparently absent, and it would seem possible that in some attachments they were absent altogether and the muscle was joined directly with the epithelium. This condition is clearly true in some lower Crustacea, and has been figured by Schneider in *Branchipus*.

Study in this connection: the mode of attachment of the closing muscle to the shell in a *Unio;* the attachment of an *Anomia* to a rock and the attachment of muscles to the head-cartilages in the squid or *Octopus*.

Technic. — Use the same methods that were used for the simpler tensile forms.

LITERATURE

Read the same articles that were suggested for the preceding parts.

THE SUPPORTING CONNECTIVE TISSUES: SPECIALIZED RIGID FORMS

We shall consider as specialized forms of rigid connective tissues, all those cases in which a large number of cells work collectively to produce a single supporting structure or homogeneous supporting material for the body. Such materials are usually produced inside of the body by mesodermal tissues and form an endoskeleton. As examples of such, we shall study the tissues that form the skeletal structure of the common sponge, others that build up the cartilages of the squid and the vertebrate animals, and those that form the bone of the mammals. Many integumental structures are, in effect, skeletal. We therefore refer the student to Chapter XX, Part A, to read, in this connection, the descriptions of the shell of an arthropod and the scale of a teleost fish. The "pen" or shell of the cephalopod mollusk, while an ectodermal product, functions purely as an internal organ of support and will be treated of in this chapter.

The Cellulose Skeleton of *Euspongia* (Fig. 68). — In this sponge the skeleton consists of long, curved, and branching rods of a material that resembles cellulose and is variously branched and arranged to loosely support and uphold the soft and delicate tissues of the body.

The particular cells that are responsible for the production of this fiber are part of the middle layer of connective tissue, and are indistinguishable from their fellows until a fiber is to be made in their neighborhood.

Then they accumulate in the path of the future fiber and its branches, and secrete and pour out the material of which it is formed. While the fiber is growing, they remain attached to it, forming at this time an epithelium-like layer of cells around it. They differ in shape and other features from other mesogloea cells during this period of fiber production, but when it is finished they retire and again assume the form of the other connectivet-issue cells.

FIG. 68. — Part of a newly forming skeletal fiber of *Euspongia*. (From SCHNEIDER after F. E. SCHULTZE.)

Shell of Loligo Pealii.—A characteristic organ of the mollusca is the shell, a thick layer of chitin, usually reënforced with carbonate of lime, that is developed on all, or a part, of the mantle.

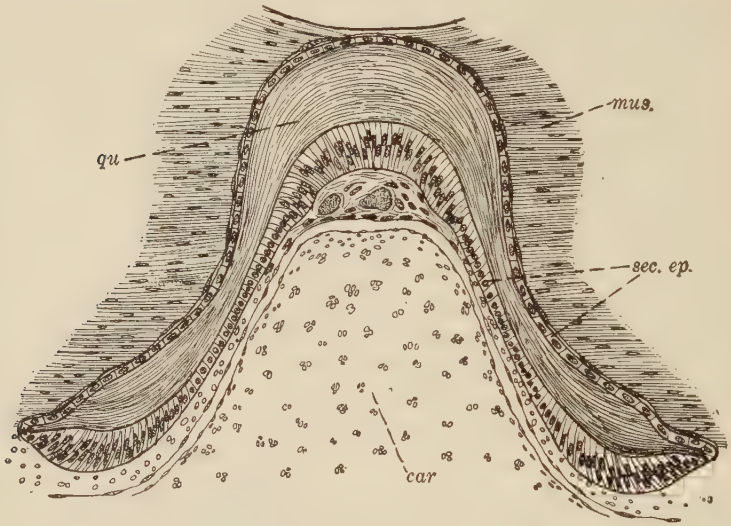

FIG. 69.—Transverse section through quill (shell) of a squid, *Loligo*. *qu.*, quill; *sec.ep.*, secreting epithelium of quill; *mus.*, muscle of mantle wall; *car.*, cartilage.

In the squid this "quill" is carried inside the body by an invagination of the surface on which it lies (Fig. 69). The formative epithelial cells, here a simple columnar epithelium, surround this shell, which is of chitin alone without any lime, and build it up from all sides. Its beautiful ribbed structure makes it an ideal organ of support. It is laid down in layers, as are all mollusk shells. At the points where it is thickest the cells that form it are also longest, and form, in consequence, the thickest epithelium.

Another kind of rigid connective substance is found in the interior of the body in many animals which is a mass of material formed by many cells working jointly. The tissue is called *cartilage*, and the substance formed by its cells is composed of several organic materials.

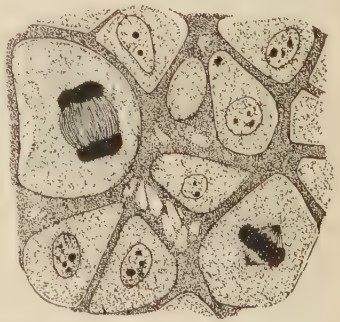

FIG. 70.—Developing cartilage of a young squid. Mitotic figures very abundant. × 1200.

The cartilage found in the cephalopod mollusks shows all sides of the development and structure of this tissue, and will represent the cartilages in general (Fig. 70). It begins as a loose collection of mesoderm

cells with large nuclei and small cytoplasmic bodies that are fairly compact but yet send out numerous branches which anastomose with one another. The deposition of the cartilage substance, called the *matrix*, begins at the principal boundary of the cytoplasmic body, and the material first appears as a thick rim of a hyaline substance surrounding each cell. These rims coalesce with each other and new concentric layers are laid down, always next to the cell and by the agency of its cytoplasm. The material of the matrix changes in chemical reaction as it grows older, and in some forms of cartilage the concentric layers of which it is composed are plainly visible. Usually they are not so unless by special treatment under the microscope.

In most cartilages, and especially in that of the squid, which we are studying, the layers of matrix do not entirely shut in the cell. The processes of the cytoplasm pass through them and unite with the similar processes from other cells, thus forming pathways from cell to cell through the matrix, for the passage of food and other materials which are carried by the protoplasmic processes.

As the cartilage grows, its cells multiply in order that there may always be enough cells scattered through the matrix to have full control of its growth, support, or atrophy at all times. This multiplication is by mitosis, although amitosis possibly occurs in later growth processes (Fig. 71), and when the two daughter cells are completed, they continue the formation of the matrix as before, from every part of the surface of their cell bodies.

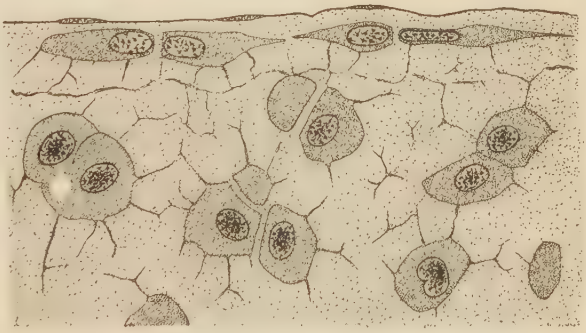

FIG. 71. — Cartilage of an adult squid. Intercellular canals partly shown. × 650.

This results in the formation of a thin but growing plate of the matrix between them, which is continued. As several divisions usually occur in succession, the cells are often found arranged in groups of two, four, or eight, as in Figure 71. The cartilage cells store glycogen, and when prepared for the microscope are usually shrunken by the loss of this glycogen, which readily dissolves in the fluids used to harden the tissue. This simple cartilage is one of a large group of tissues of which there are several kinds.

The simplest kind is that just described, and is known as a *hyaline*

cartilage. A variety in which elastic fibrils have been developed in the matrix is known as *elastic* cartilage; and still another, in which white tendon fibrils have penetrated the hyaline matrix, is known as *fibrous* cartilage.

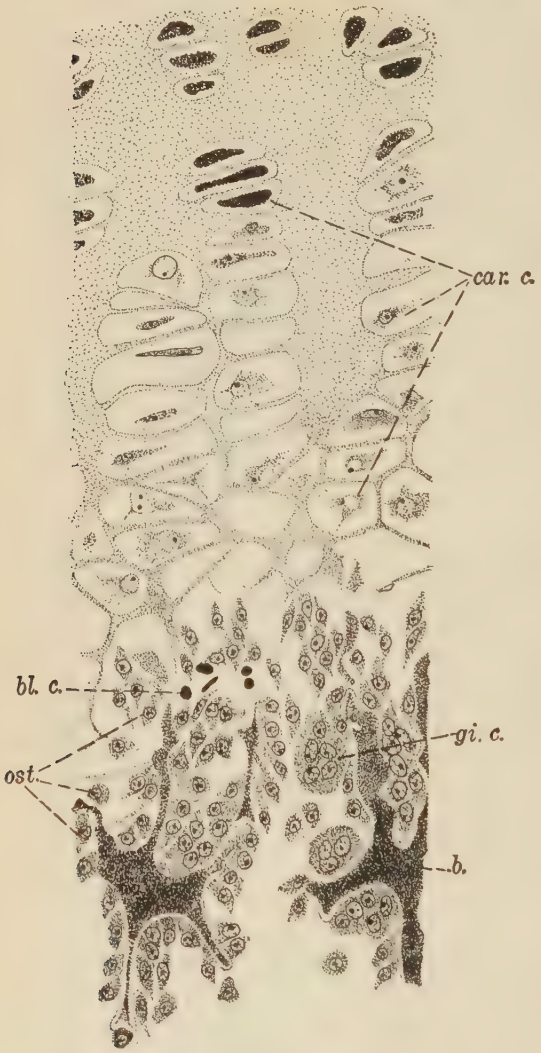

FIG. 72.—Reconstruction of cartilage into bone. *car.c.*, cartilage cells in successive stages of degeneration; *ost.*, osteoblasts; *gi.c.*, giant cells or osteoclasts; *b.*, young bone; *bl.c.*, blood cells.

The bone of the vertebrates is perhaps the most highly developed and efficient of the rigid connective tissues. It is formed from the mesodermic tissues and is always an internal structure. It has many varieties in the groups of chordate animals, and we shall select the best-known form as an example, the bone of the mammals and of man.

This structure is formed in two ways, producing two slightly different structures. The most primitive, perhaps, is the *endochondral* bone, formed by the transformation of hyaline cartilage (Fig. 72). The second is formed directly from the connective tissue, especially that which surrounds a cartilage or a bone. This is known as *perichondral* bone formation. It can be formed in the connective tissue quite apart from any cartilage. Endochondral bone is formed as follows in an embryonic finger cartilage.

The bone transformation begins in the middle of the joint and advances as a curved line in both directions, distad and proximad. This line is the point at which the cartilage may be said to change into bone and, as it moves, the cartilage cells in front of it go through a series

of changes, beginning when it has arrived within a certain distance of them. Also when it has passed it does not leave fully formed bone behind it. Only when it has passed a certain distance does the tissue finally appear completed. The process thus occupies a zone of some width. This movement is called the line or wave of ossification. At the point where it begins a blood vessel loop enters the future bone, bringing with it the various bone-making cells of the perichondrium and a blood supply for circulation.

As the line of ossification approaches within a certain distance of any point of the cartilage, the cartilage cells occupying that point begin to change in form as well as position. The change in position is most marked (see Fig. 72). Instead of lying scattered at random with only a somewhat regular proximity of the daughter cells of common ancestry, the cartilage cells arrange themselves in more or less regular rows placed at right angles to the line of advancing ossification.

How the cells move in order to place themselves thus is not known. As the spaces that they occupy in the matrix become wider and narrower as well as larger than before, and as the increasing size of this space shows that the cartilage cells eat away the matrix, it is probable that they move by dissolving the matrix before them. Once in line, the dissolution of matrix goes rapidly on, and, as the cell spaces are nearer to those next them in the line than to those in the neighboring lines, it follows that the spaces in each line break through their barriers and form channels with irregular strands of matrix lying between them. Sometimes two or more channels will unite to become one larger one. The cartilage cells, meanwhile, slowly swell up, lose their staining power, and by the time the spaces are broken into, have entirely disintegrated.

When the process first began at the center, a "bud" of the perichondral membrane, a connective tissue covering the cartilage, was evaginated into the space left by the first dissolution of matrix, carrying with it a loop of blood vessel and its own inner layer of modified cells, that have the power of secreting various salts of lime and depositing them on whatever surface they may rest against.

A layer of these cells is pushed up into each of the channels and against the surface of its walls, the remnants of the cartilage matrix. Here they begin the deposition of lime salts, the materials of which are supplied them by the loop of blood vessel that grows into each channel. These vessels follow closely behind the perichondral cells which, in their new position and function, are called the *osteoblasts* or bone-making cells.

The osteoblasts lay down the lime salts, first in the remnant of cartilage matrix and then in successive internal layers. Between each neighboring pair of layers some of the osteoblasts are left in small chambers of the bone called *lacunæ*. In this position they are called *bone cells*.

Provision is also made, by fine channels called *canaliculi*, for the processes of the bone cells to reach and anastomose with each other, thus forming radial pathways for the transportation of food and other matter from the central canal, or Harversian canal as it is now called, to the entire group of bone cells between the surrounding lamellæ of bone. The whole structure, central canal containing blood, nerve and reserve bone cells, together with the surrounding lamellæ and the bone cells between them, is called an *Harversian system* (Fig. 73).

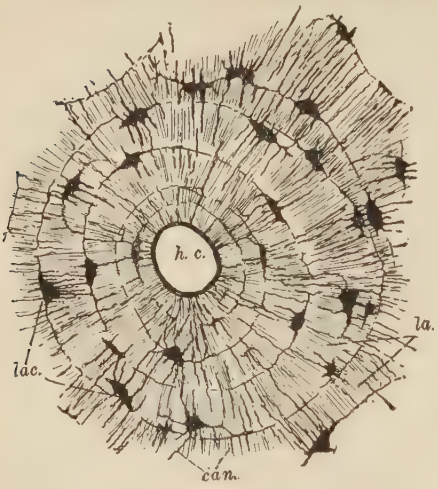

FIG. 73.—Transverse section of a Harversian system of bone. *h.c.*, Harversian canal; *can.*, canaliculi; *lac.*, lacunæ; *la.*, lamellæ.

Bone is made up of a mass of these structures formed not only by the transformation or, to be more accurate, the reconstruction of cartilage, but also by the activity of the membrane surrounding the bone, the *periosteum*, which is the same as the *perichondrium*. This periosteum is made up of several connective-tissue layers, the inner of which are modified into compact cells of some size, the osteoblasts, which are the same as those that entered into the cartilage to replace its matrix with the bone substance.

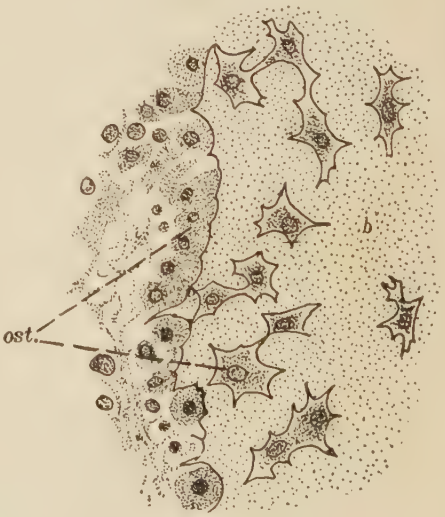

FIG. 74.—Formation of bone by the periosteum. *ost.*, osteoblasts laying down bone and becoming inclosed in this bone as bone cells; *b*, bone substance. (Part of a figure by LEWIS from STOHR'S Histology.)

Where this membrane is in contact with the surface of a bone, such as the one whose reconstruction from cartilage we have just described, its inner layer of osteoblasts begin to lay down bone material (Fig. 74). They do not do so in simple layers but in long, hollow grooves whose edges are built up more rapidly than the rest, finally meeting and inclosing a part of the osteoblasts and a

core of connective tissue containing a blood supply. This core then operates with its peripheral osteoblasts to form an Harversian system, just as the osteoblasts did in the cartilage channels during endochondral bone formation. The rising edges of the future Harversian systems are laid down in a connective-tissue basis that precedes them. This is shown in Figure 74.

One other histological process in connection with bone formation should be studied here. As most bones increase in diameter, they become hollow in the center, this space being filled with the marrow. In order that this may occur, some of the bone material must be removed, and this occurs as follows.

Large, heavy cells appear in the Harversian canals and, applying their bodies to the bone material, they proceed to absorb it and remove it. They probably do this by secreting some acid in the cytoplasm and dissolving the salts, which are then carried away by the blood. It would be interesting to know more about the acids that are used and what becomes of the salts in solution; they may be used over again by the osteoblasts. These large cells that remove the bone substance are called the giant bone cells or *osteoclasts* (see Fig. 72, *gi.c.*). They usually contain several nuclei.

Technic. — The technic of this group of tissues is somewhat difficult on account of the hardness of some of them. To see bone properly, one should study both decalcified and ground sections of the tissue. Developing bone is easily cut in both paraffin and in celloidin when the fixing fluid has been an acid one like Flemming's fluid and most of the others. Any of these tissues may be first fixed and subsequently decalcified with nitric acid in connection with phloroglucin (see Lee). Some of the shells must be ground, and then the soft parts are nearly always destroyed. Many of the tougher integuments may be cut, at some expense in the sharpening of knives.

LITERATURE

Read the general literature on the simple rigid forms and then see the descriptions of bone and its histogenesis in some good medical histology.

FAT

Various kinds of cells can store up prepared food materials in their cytoplasm as a reserve supply in the economy of the animal. Chief among these are the otherwise unspecialized connective-tissue cells of the vertebrates that are able to take such materials into the cytoplasm in the form of various fatty acids. These are known as the *fat cells*, and

they are found among practically all of the mesodermal parts of the body, acting also in some degree as buffers and fillers for otherwise unoccupied spaces. Their content is often called upon for food when anything goes wrong with the other means of nourishment, and they give up this substance readily to the blood to be carried where it is needed.

FIG. 75. — Developing fat in subcutaneous tissue, showing fat vacuoles. (After LEWIS.)

The fat of the mammal may be best studied by tracing its genesis in the embryo (Fig. 75). We shall do this in the human embryo, beginning with sections of the skin from a fœtus of between four and five months' development. These cells are at first exactly the same as the other connective-tissue cells lying around them. When they begin to differentiate, a number of tiny droplets of the fatty substance appear in their bodies, and as these droplets grow in size they push the nucleus to one side. In growing, they merge together until they form but one large drop. For some time after the large drops of fat are formed, other small and new drops arise in the cytoplasm, and later join the large single drop, until, at the maturity of the drop, it is many times the size of the original cell and shows the cytoplasm of this cell lying around it as a thin cover, thickened enough at one side to contain the nucleus. Enough of the undifferentiated connective-tissue cells remain in the tissue to hold it more or less firmly together. Figure 76 is drawn from the integumental fat of the chicken in which these relations are particularly favorable for observation.

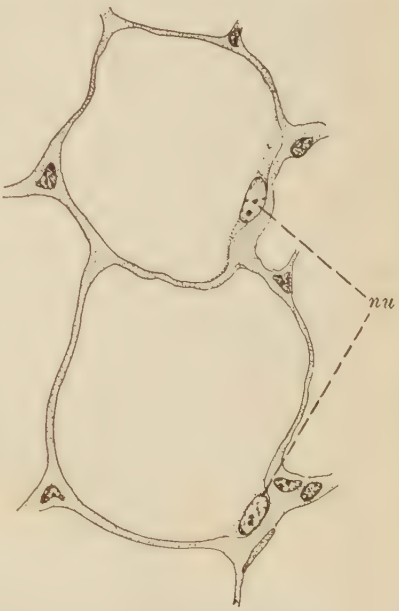

FIG. 76. — Fat cells lying in connective-tissue reticulum. Skin of chicken. *nu.*, fat cell nuclei. × 870.

There are many cells in the invertebrate forms that store up prepared food materials. In no case, however, is this material exactly like the vertebrate fat in chemical composition. In some cases it more nearly

resembles the materials stored in the vertebrate liver. The cells, however, seem to store them as reserve food supplies, as is demonstrated by the fact that before particular growth periods, when the creature will be unable to eat, huge quantities of these materials are collected in the cells, and when the growth and molting is over they have been used.

FIG. 77. — Cells from fat body of the insect, *Regalis*. × 580.

The "fat-bodies" of the insects furnish an example. These bodies are strings of mesodermal cells lying in the body cavity of various larvæ. Figure 77 shows a section of such a fat-body from the larva of a *Regalis* moth. This larva had just completed a molt, and the cells do not therefore show as much food material as they would if the tissue had been taken just before the molt.

The Crustacea also present analogous tissues that are probably used as stores of reserve foodstuffs, especially just before the molting period.

Technic. — The fats may be cut like any other tissues, and such sections yield very good results, especially when one is already well acquainted with the tissues. To get so acquainted as well as to decide with accuracy if a given vertebrate tissue contains fat or not, the material should be cut fresh with a freezing microtome and the sections stained with some of the specific fat stains, of which there are a number in the list of analine dyes. Osmic acid will also stain the fat substance a good black that can be readily recognized.

LITERATURE

KOSCHEVNIKOFF, G. A., 1900. "Über den Fettkorper und die Oenocyten der Honigbiene (*Apis mellifica*)," *Zool. Anz.*, Band XXIII.

WIELOWIEJSKI, H. v., 1883. "Über den Fettkorper von Corethra plumicornis und seine Entwicklung," *Zool. Anz.*, 1883, VI Jahrgang, pages 318–322.

Read of the development and structure of human fat in any of the best medical histologies.

CHAPTER VIII

THE TISSUES OF MOTION

MOTION is an almost universal attribute of protoplasm. Practically all cells can move some part of their body, even if they have no means of moving the body as a whole from place to place. Thus, the motion may consist of internal operations, as circulation, ciliary movement, or the many and varied acts of cell division.

Or it may be exhibited as the *contraction* and relaxation of the cell-body as a whole, as in some epithelial and other cells. Usually the act of relaxation, as performed by elastic and other non-living parts of the cell, is supplanted by another motion in a different plane, which acts as a counter to the first movement, restoring the mass to its previous form and shape.

In some free cells, as *Amœba* and the white blood corpuscles, these movements result in complex flowing and creeping motions that move the entire cell from place to place. In other unicellular forms the more or less numerous cilia or flagella on the body, which is rigid, move the creature in any direction and at high rates of speed. Both of these forms of movement are found in the simply organized and small unicellular animals, but in the great majority of higher form this function of moving is all performed by special cells, only, of the organism, and these cells present as high and complete a specialization as we find in nature. We shall call them the *muscle cells*. The cytoplasm of muscle cells is of characteristic granular structure and is called *sarcoplasm*. The granules are known as *myochondria*.

The fully specialized muscle cell can contract with a force, rapidity, and quick succession far beyond the power of less specialized protoplasm. It gets this greater power and efficiency from the development in its cytoplasm of certain long thread-like regions of contractile substance, the *muscle fibrils*. These fibrils are the cell-organs of contraction and vary in appearance and number according to the needs of the tissue. They are formed as plastid-areas of the sarcoplasm.

They lie parallel to the long axis of the cell and in the direction of motion. They vary in the completeness with which they are differentiated out of the sarcoplasm of the muscle cell. They also differ in structure so that they may be classed as striated or non-straited fibrils. This

striation or segmentation consists of a transverse division of the body of the fibril into a larger or smaller series of equal working units called *muscle elements*, *sarcous elements*, or *sarcomeres*, and these have a definite structure which is exactly the same in all the fibrils in a given cell. Each sarcous element itself has a definite and symmetrical segmentation which varies in different forms, and changes during the contraction and expansion of any given example. This shall be described in detail further on.

The non-striated fibrils are perfectly smooth. In some mollusks a peculiar woven appearance of the fibers is probably due to the irregular arrangement of its weakly striated fibrils.

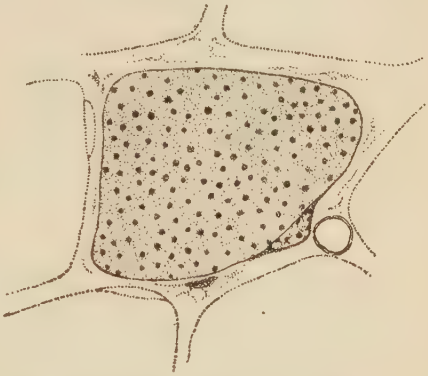

FIG. 78. — Transverse section of a striated muscle fiber from the bat's tongue. To show the comparatively great amount of sarcoplasm. × 1200.

Some muscle cells may contain but few of the fibrils scattered singly through the sarcoplasm. Again, in others, the fibrils are more numerous and are gathered together into a number of column-like (see Fig. 92, lobster's cardiac muscle) or plate-like groups (see Fig. 82). These groups will be spoken of as *fibril-bundles*. When cut in transverse sections they are known, in mammalian muscle, as *Conheim's fields*. In the bat the fibrils are all separated and are not so grouped (Fig. 78). Sometimes the fibrillæ are so numerous and form such a homogeneous mass that they fill the greater part of the cell, only leaving room for the nucleus and a single cone-shaped area of sarcoplasm at each end of it.

Each muscle fibril is usually surrounded by a differentiated zone of sarcoplasm, which may be called the *cement substance*. This cement substance is common to all the fibrils in a bundle, in some forms of muscle (see Fig. 82). In others it is not, and in some it is not apparent at all.

Any conceptions of the cause of the mechanism of motion that we may entertain must center around the fibril. Have we here an intensification of the same phenomena that occur in an *Amœba* when it contracts, or was the first fibril a new organ that enabled its possessor to do more than the life forms had been able to do up to that time?

Engelmann has given us, so far, the best theoretical explanation of the motion of the fibrillated muscle cell. In this explanation the fibril is supposed not to have any "vital" power of moving, but to be an inert

secretion of the cell and a substance particularly sensitive to several chemical or physical laws which rule that, when warmer, the substance must absorb water, and that when such a substance absorbs water it must become thicker and shorter.

Then, assuming that the plasma of the muscle cell, which surrounds all the fibrils in it, is filled with some secretion substance that gives heat when combined with oxygen, and, assuming that the motor nerve impulse causes oxygen to unite with this substance, we can imagine the sudden warming of the watery plasma by the rapid oxidation of the heat secretion and the sudden shortening of the fibrils by the absorption of water.

This explanation does away with all unknown vital factors, except a secretory activity of protoplasm that enables it to produce the easily oxidizable material, and a nerve stimulus that can cause oxygen to suddenly unite with this substance. The secretion power and the nerve stimulus remain to be explained.

Another but less satisfactory explanation of more remote causes is Schaefer's theory whereby the isotropic or light substance is supposed to retire into a series of longitudinal channels in the anisotropic substance, thus swelling and shortening the latter. This process involves electrical and chemical changes. It is not as clear as the first.

The cell-membrane which surrounds a muscle cell is known as the *sarcolemma*. In a large number of forms this membrane is reënforced by the closely applied bodies of connective-tissue cells, in which case there has been no change of name and the entire covering is still called the *sarcolemma*. In most cases it is practically impossible to demonstrate a sarcolemma apart from this connective tissue.

A muscle cell is always in bodily connection with a nerve cell which controls it. The organ by which this contact is effected is known as a motor nerve ending.

The muscle cells are of several shapes (Fig. 79). Spindle shape and tapering to pointed ends (*D*), elongated cylinders with blunt ends (*B*), cubical cells arranged end to end (*A*) are the shapes usually found, while epithelial cells with the base flattened out and converted into muscle (*C*) and branching cells are some of the unusual forms. The spindle-shaped, cubical, and epithelial cells are usually mononuclear, while the cylindrical are nearly always multinuclear cells or syncytia.

In many small and simply organized animals the muscle cells are placed apparently at random through the tissues of the body or in layers on its surface. At the point where the animal attains to developed powers of movement, however, these scattered fibers become localized into groups which are placed in positions where their force can be exerted to the best advantage. Such groups are called the muscles. They can operate to move the body in two principal ways. The first is *without*

the use of a rigid and jointed skeleton, and the second is *with* such a support. To understand how the first method can be used to produce rapid and accurate motion, study the use of muscle in the arm of a squid or octopus, or the proboscis of an elephant. The action of muscle with a jointed skeleton, is doubtless already understood by the reader from seeing figures of this operation in man and in a bivalve mollusk. As the structure of muscle systems that operate without a skeleton is often

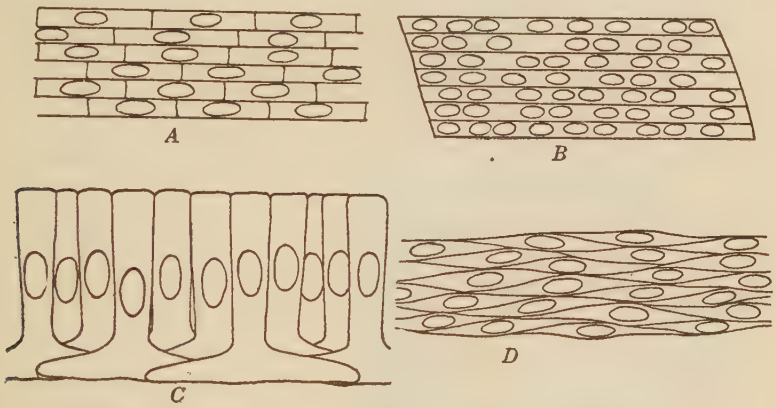

FIG. 79. — Diagram of several ways in which muscle cells are arranged to form muscle tissue.

a subject of histological study, we shall **briefly examine an example of it in a squid's arm.** Figure 80 shows a line-drawing of a transection of this organ, and the lettered bundles of muscle are used at this point as follows.

All muscles cut in cross section can be used as retractors to shorten the arm. The most powerful retractors are seen to be in the outer part of the mass. They are also used for the partial contractions that bend the squid's arm. The method of extension is more difficult to see. There being no rigid frame, the only method is to use some lateral compressions which will force the mass to extend in the direction of the arm's long axis and at right angles to the plane of the figure.

An examination of these lateral muscle bundles will show that they are confined to a circular, central core, sharply defined from the outer layer of longitudinal fibers by a sheath of delicate connective tissue. This core contains some small bundles of the longitudinal fibers, but the greater part of its mass, outside of its non-contractile nervous ganglion in the center, is occupied by fiber bundles that lie in all possible directions in the plane transverse to the long axis of the arm. When these muscles contract, the central core is compressed laterally and consequently extended longitudinally, while the outer tissues are, of course, extended with it.

The extensory core is placed at the most efficient point in the section, at the center. Not that the outer edge would not serve as an extensor just as well, but because this outer edge must be reserved for the bending muscles, which serve to bend, wave, and curl the pliable arms in all directions. They can do this, too, independently of the state of extension or retraction of the arm, owing to their independence of the core. Watch a live octopus or squid and see that its arm can twist and curl, extend, or contract in practically any direction. All these movements are due to *contractions* of muscle cells, for no muscle cell can extend itself. This must be done for it by some other counteracting muscle cell properly placed to oppose it.

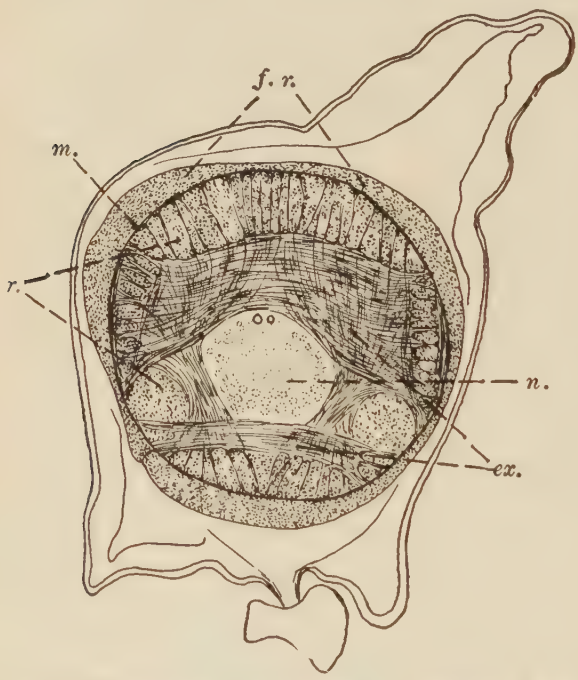

FIG. 80. — Transection of squid's arm; *r.*, retractors; *ex.*, extensors; *f.r.*, flexing retractors; *m.*, membrane separating the extensor core (with its retractors) from flexors or bending muscles; *n.*, central nerve cord. × 80.

Study the action of the muscle layers in the intestine of vertebrates also. For the origin of the muscle tissues, see later in this chapter.

Several features have been used to classify muscle cells, but no two of them agree except within very narrow limits. Their control by the will divides them into voluntary and involuntary muscle cells, the markings of the fibrillæ serve to classify them as striated and non-striated muscle cells, the number of their nuclei makes them mono- or multinuclear, and less important features group them into incomplete classes, as epithelial, branched, and circular muscle cells. Our study may well begin with the cylindrical, multinucleated, striated fiber. Such a muscle cell is universally found in forms of all grades where efficiency and economy is needed. It is of high specialization and is under control of the will. The example taken is from the common brook sucker, *Catostomus communis*.

Muscle of Adult Sucker. — A superficial examination shows that the entire muscular body-mass of this fish is composed, on each side of the spine, of a series of regularly bent plates or myotomes, fitting closely to one another and joined surface to surface by layers of connective tissue, or *septa*. The myotomes lie, as far as their shape allows, at right angles to the body axis.

Under the low power it will be seen that each plate or myotome is a mass of fibers which stretch from surface to surface, and which lie exactly parallel with the body axis and consequently with each other. They thus are frequently attached to the septum at a small angle.

Under the high power (Fig. 81) each fiber appears at first sight to be composed of a series of thread-like and regularly marked structures, the fibrillæ, which run parallel to each other the entire length of the fiber. Still closer attention will show that this mass of fibrillæ does not alone constitute the fiber, but that it occupies the larger part of the real fiber, which is a mass of sarcoplasm containing nuclei and bounded on its surface by a thin, tough membrane, the sarcolemma. The sarcolemma adheres closely to the entire surface of the fiber.

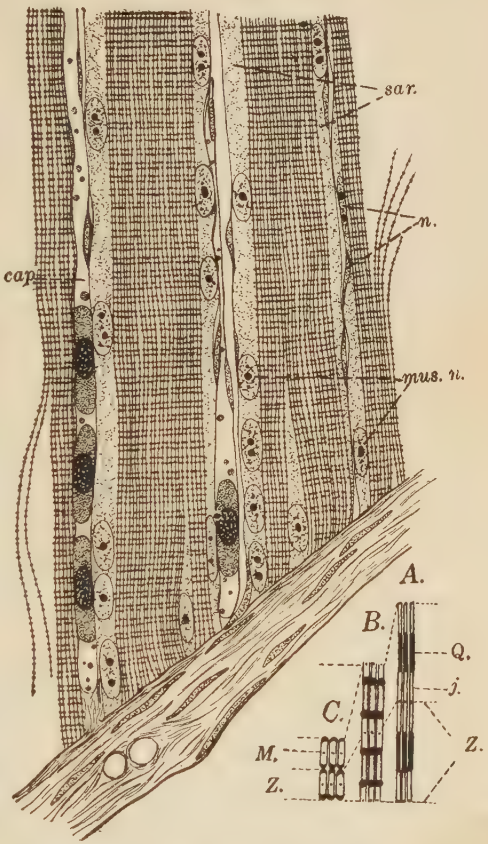

FIG. 81. — Longitudinal section of a bit of muscle from the sucker, *Catostomus*. As the scale will not permit of fine detail, a semidiagrammatic sketch in the lower corner serves to show the relations of dark and light elements in the three contraction stages of the fibril. The chief figure shows the *Q*-stripe separated as in *B*. *cap.*, capillaries containing blood cells and blood platelets; *sar.*, sarcoplasm; *mus.n.*, muscle nuclei; *n.*, connective-tissue nuclei. × 1000.

The sarcoplasm fills the entire long cylindrical muscle cell and, besides the prominent bundles of fibrillæ which lie in it, there are also many nuclei of a specific character which distinguish them as muscle nuclei when compared with any other nucleus in their locality. They are

slightly elongate, larger than the average nucleus in other tissues of the sucker, and have a single or double nucleolus of large size that stains jet black in this specimen. The nucleoli lie in the longer axis of the nucleus, and the caryoplasm is clear, owing to the small amount of the chromatin which gathers into a few dense masses near the nuclear membrane.

The nuclei are fairly numerous and generally lie in the cytoplasm between the fibril bundles and the sarcolemma. They here form rows that stretch for some distance in the fiber on its long axis. In addition to these muscle nuclei lying outside the fibril bundles there are several which lie inside or among the bundles. These are always placed close to the extreme end of the fiber, near the septum of the myotome, and the fibrillæ part to pass on each side of them, leaving a long cone-shaped space at each end which is occupied by sarcoplasm. The sarcoplasm is remarkable for its granular rather than its reticular appearance. This is due to the large number of granules, the myochondria, which lie in irregular masses in an otherwise typical cytoplasm.

A delicate lax connective tissue is found throughout the mass of muscle fibers. Its individual cells, whose outlines are irregular and indeterminate, possess nuclei which are characteristic, being thin and flat and round, or elongated into an oval form. They are smaller than the muscle nuclei and possess a denser caryoplasm, which contains more chromatin. The nucleolus is multiple and its several very small parts are distributed around the periphery of the nucleus rather than in the center.

Such portions of this network of connective tissue as come in contact with the cell body of a muscle fiber, form a layer that covers every part of the fiber's surface and is so intimately connected with the sarcolemma that it cannot be separated from it. The true sarcolemma is a cell-wall of homogeneous structure and of no great strength or substance. It cannot be demonstrated apart from the connective-tissue layer which surrounds and adheres to it. Such nuclei as are to be in the sarcolemma belong, of course, to the connective-tissue elements.

Blood capillaries, with their delicate endothelial walls and contained red corpuscles, lie between nearly every two fibers. The nuclei of their walls are almost identical in shape, size, and other features with the connective-tissue nuclei. Blood plates are well demonstrated in these capillaries.

The bundle of fibrils next claims our attention. That this is composed of real individual fibrils is indicated by its appearance and by the fact that in the specimen we examine, some individual fibrils are separated from their fellows and shown alone and in their integrity.

Such a fibril is composed of two kinds of substance according to the

staining and refracting power, and these materials are distributed with absolute regularity and evenness. One of them, the so-called *isotropic* substance, which does not readily stain, is probably responsible for the tensile continuity of the fibril and is also, probably, a less specialized form of cytoplasm than the second, or anisotropic substance, which is doubly refractive and stains in our subject a jet black.

The anisotropic substance is deposited, in resting or relaxed sucker muscle, in regular areas of the fibril, which areas are of equal length and spaced equally apart. Each of these areas is designated for convenience by the capital letter *Q*. Such a portion of anisotropic substance, together with one half of the isotropic substance on each side of it, is called a *sarcous element* or *sarcomere*. This same arrangement holds for all the other fibrils in the sucker's voluntary muscle, and where a number of fibrils are grouped together in a bundle, the sarcous elements are all in perfect alignment and directly opposite one another. This gives the fiber a banded or cross-striped appearance, from which it gets its name, striated muscle (see Fig. 81). These broad black stripes, as shown in a portion of resting sucker muscle, are almost exactly two thirds the width of the intervening light stripes.

In most instances this band of the sarcous element is divided at its middle by a lighter band. This is caused by the beginning of the physiological act of contraction in which broad black bands of the sarcous elements separate into two parts. Figure 81, A, shows this condition.

Looking closely at the light stripe which lies between the broad median band of the sarcous elements, we see that it is divided midway by a black line into two equal parts. This line we shall call the *intermediate septum* (*Krause's membrane*). Referring to one of the component fibrils again, it is seen that this intermediate septum is represented in the fibril by a dot or spot which may be called the *intermediate granule*. A very close inspection under high power will probably show that a transparent membrane connects the various intermediate granules into one plane, and the whole structure forms the intermediate septum, which has been called *Krause's membrane* from its discoverer. This is very difficult to see in the sucker muscle, and may be much better observed in our next specimen, the lobster's muscle. Turning now to a section taken at right angles to the sucker's muscle fiber (Fig. 82), a number of points of interest can be made out to corroborate impressions formed while examining the longitudinal sections.

The fibers are here seen to be of several sizes, and the continuous outline of each sarcolemma can easily be made out. The connective-tissue nuclei of the sarcolemma are sharply distinguishable from the muscle nuclei which lie in the sarcoplasm of the fiber between the sarcolemma and fibril bundles.

These bundles take up the greater part of the fiber, and the sarcoplasm lies between the groups of fibrillæ, into which the fiber bundles are divided. This grouping of the fibrillæ is clearly seen in the cross sections of sucker muscle. The fibrillæ are grouped, near the periphery of the fiber, into plates of one fibril in thickness. The form of the plates is due to the presence of a mass of heavier cytoplasm that surrounds and binds them together. This is the *cement substance*.

These peripheral plates extend for the same distance into each fiber, whether it be large or small, and inside of that the fibrils are no longer

FIG. 82. — Transection of several related fibers of muscle in the sucker *Catostomus*. *f.*, smallest fiber.

a part of the peripheral plates, but are either placed singly or grouped into smaller independent plates. Thus in the smallest fibers (as at Fig. 82, *f.*), the plates extend to the center, while all larger fibers have the center made up of detached groups. It seems that the fibrils are thus placed in thin layers side by side, in order that each and every fibril may be in direct contact with some part of the sarcoplasm from which it draws its food and stimulus, and on which it unloads its refuse materials.

That the plates do not extend in a complete condition further inward than they do is probably due to the requirements of the movement of the fiber which does not contract and expand all its parts in perfect unison, as will be shown elsewhere.

The individual fibrils which show so distinctly in the longitudinal sections cannot be ordinarily seen in a transverse section of the fiber. This may be due to the optical properties of the cement substance which surrounds each fibril and which binds them together in the plates. The

plates appear homogeneous in section, although at one point it appears that the fibrils are separately and distinctly shown in several of the plates.

A closer study of the structure and relations of the fibrils should now be made in some muscle whose elements are more favorable for a detailed observation. A suitable muscle for this purpose can be found in **longitudinal sections of the muscle in a lobster's limb joint**. Figure 83 is from the basal joint of the antenna in a lobster that was on the point of emerging from its old shell.

This figure represents a part of a fiber, the upper end of which was attached to the shell. The two smaller nuclei are probably the first edges of nuclei that are, in reality, as large as the two larger ones.

The fortunate condition is that the part of the muscle next to the shell was at rest and in a relaxed condition, while all the other segments showed successive stages of contraction. The first segment measured, in one of its magnifications, $17\frac{1}{2}$ mm. Each succeeding one measured a little less, until the last is only 12 mm. long. This alone shows us that we have a carefully graded series of contraction stages, and an examination of the appearance of the successive stages confirms this view.

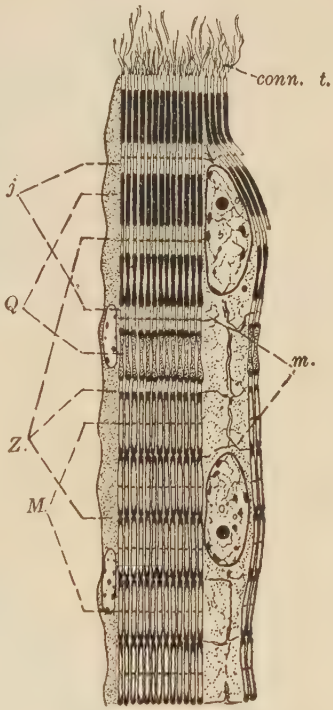

FIG. 83. — Bit of a muscle fiber from a lobster's antenna. Partly contracted. Lettering of unit regions same as in Fig. 81. *m.*, delicate membrane extending through cytoplasm from all *Z* planes. Apparent difference in size of muscle nuclei due to tangental sectioning of the smaller ones. *conn.t.*, connective-tissue fibrils which attach the muscle fibrils to the hypodermal cell fibrils.

In the first upper segment, of the eight sarcous segments shown in any one of the sixteen myo-fibrils that compose this fibril bundle, we can notice that the anisotropic material is deposited in a long rod-like area in the middle of the segment. This area we will designate as *Q*, and it is usually so called in works on muscle structure.

On each side of *Q* is an area of non-staining isotropic substance not more than a quarter as long (or wide, if we consider the whole band that they form) as *Q*. Conforming with usage, we shall call this *j*. There are two of these.

Bordering each *j* on its side farthest from *Q* is a row of dots or round

bodies. These are of black staining anisotropic substance and are called *Z*. They mark the limit of the sarcous element or muscle unit of the fibril, and where two elements touch, the dot is double, although the two parts are molded together and do not usually appear as twin bodies. The upper segment in our figure shows both a double *Z* body (where it touches the segment next lower) and a single or half *Z* body at its upper end, where it is attached to the connective-tissue cells.

The *Z* bodies, then, form the intermediate line and are connected with one another by a transparent or non-staining membrane that extends through the cell from side to side, even in such parts of it as contain no myo-fibrils (see Fig. 83, *m*.). This can be well seen in the portion of the fiber figured, especially where the larger and smaller bundles of fibrils have separated to make room for the two large nuclei and their surrounding sarcoplasm.

The second muscle segment shows no great difference from the first, although it is a trifle shorter. In the third, however, a change has occurred and *Q* has become thinner in the middle. The whole segment is about one twentieth shorter than the second. In the fourth segment the *Q* area is not only thinned out in its middle, but its substance does not stain as black in this thinned middle part.

The remaining five segments are alike in the fact that the *Q* areas are elongated until their black ends have met the corresponding ends of the *Q* areas of the neighboring segments. In doing this they hide the *Z* dots and make a much darker and wider band across the muscle fiber in its place. Their middle parts have become clear and non-staining except that an edge appears to be left on each side. This is probably a refraction line.

In the middle of this *Q* area, now light and non-staining, appears a small black dot that resembles the *Z* body of the relaxed stage except that it is smaller and is not connected with its neighbors by a membrane. This dot with its neighbors forms the *M* stripe. In Figure 84 the *M* stripe is the same width as the two halves of the *Q* stripe.

The usual appearance of the fiber is now entirely changed, and upon a careless examination appears to have been reversed, as though the *Z* bands and *Q* bands had changed places. It takes a careful eye, even in such a favorable specimen as the one from which this figure was drawn, to run from band to band and note the real change. This difficulty is a weakness of the eye muscles and can be demonstrated by an attempt to count the pickets in a distant fence, which can be seen clearly if the eye remains still, but are lost count of if the eye moves to follow them.

Many other forms of striated muscle have a more complicated pattern of sarcous element than the ones we have been studying, as in some in-

sects, for instance, where a band of anisotropic areas in the fibrils extends through the middle of the *j* area. When found, this band is called the *N* band (Fig. 84). Its presence causes some unimportant complications in the contraction processes. The isotropic bands are designated by small letters.

In most muscles the bands are so fine and close set that it is difficult to distinguish them clearly. Then, again, one does not know which stage of contraction is presented. The usual condition shows one of the intermediate stages, as in the sucker muscle, where the eye of the ordinary observer would take each of the two ends of the divided *Q* stripe for the *Q* stripe of a resting muscle, until study showed the true relations.

Some sure way of fixing a bit of muscle so that it will be certainly either relaxed or contracted would be a useful method. A muscle fixed under pressure or relaxed does not necessarily show its fibrils in either condition. The hardest thing to get is a fiber in which there is a slow contraction wave showing as in Figure 83. Many sudden and contorted changes that are almost useless for study are usually found in crustacean muscle fixed in the ordinary reagents.

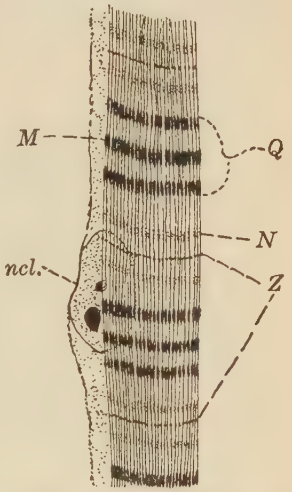

FIG. 84. —Bit of muscle fiber from the body-wall of an adult larva of *Corydalis cornutis*. Shows the (*N*) stripe near the Krause's membrane (*Z*). *ncl.*, nucleus.

Technic. —The element of "luck" appears to be a large one in the preparation of striated muscle tissue. The best histological methods, when most carefully carried out, are pretty sure to give bad results from the point of view of him who wishes to study the stages and processes of contraction. An even more careful study, than has been made of the conditions under which muscle may be killed so as to show any particular stage, is most desirable. Muscle which has been allowed to die a natural death and which has been killed with chloroform and in many other ways should be examined. Animals should be killed with poisons of marked muscular reactions and the condition of this tissue noted. The tissue can also be studied, to a certain extent, while it is yet alive.

LITERATURE

The works of Rollet, Engelmann, Retzius, and others should be read by those who wish to go further into the subject.

THE HISTOGENESIS OF STRIATED MUSCLE

Most striated muscle is developed from some sort of external or internal epithelium, the fibrils being formed *in situ* in the periphery or on one side of a columnar or prismatic cell, which may have many positions in the body.

In the medusa it is the external, covering epithelium that develops the myo-fibrils. These fibrils are formed in the expanded bases of the cells. The cells may lie on the surface or they may be invaginated into grooves, and only the inner cells of the groove have the muscle characteristics, as in Figure 85. This represents a transection of three such invaginated grooves in the epithelium on the upper surface of the body of a Florida medusa, *Cassiopea xamachana*. It will be noticed that only the one or two cells lying in the bottom of the groove (which is closed) have developed the muscle fibrils, which may be seen in section as black dots. In a younger specimen or on the outer and weaker surfaces of this same specimen there would be no invagination, and the myo-fibrils would be seen lying in the bases of nearly all the cells.

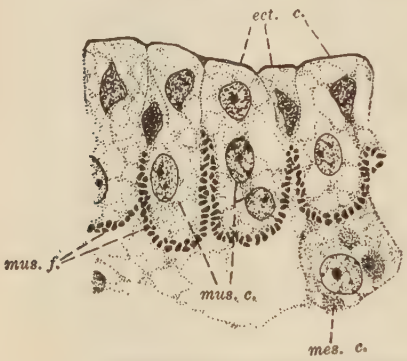

FIG. 85. — Muscle cells derived from ectoderm in *Cassiopea xamachana*. *mes.c.*, mesodermal cell; *ect.c.*, outer ectodermal cells, which probably play but small part in muscle formation; *mus.c.*, ectodermal cells which are invaginated into grooves and form muscle fibrils in their proximal cytoplasm. The grooves and muscle fibrils (*mus.f.*) are both seen in transverse section.

The vertebrate animals develop their muscle in cells that were originally a part of the epithelium that lined the primitive cœlom. These cells lose their connection with the cœlom when the epithelium, of which they are a part, is invaginated from the cœlomic surface into a number of buds, which are cut off and form a row of *myotomes* or *myomeres* in the sides of the animal. These sac-like masses flatten in a manner to form two plates and reduce the invagination cavity to a plane line. The inner plate forms connective-tissue elements, while the outer develops the striated, voluntary muscle of the body.

The development of striated muscle in the embryo fish will furnish us with a good example. We shall use an embryo sucker, which is easily procured and prepared.

A section of a very young embryo of about 2 mm. shows the

outer or myogenetic plate of the myomeres, each of which consists of a solid mass of embryonic cells with polygonal sides, fitting closely to one another and not differing greatly in appearance from the generality of other embryonic cells around them. They are rapidly multiplying at this time, as is demonstrated by the numerous mitotic figures that are to be be seen (Fig. 86).

The first evidences of muscular differentiation are most marked. The cells on the outer edge of the myotome begin to lengthen, pushing

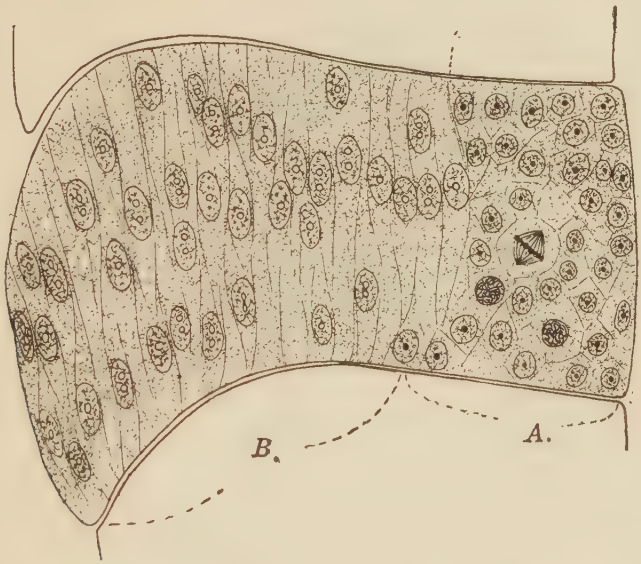

FIG. 86. — A very young myotome of an embryo of the sucker, *Catostomus*. *A*, region of mitotic multiplication of sarcoblasts; *B*, region of amitotic multiplication of nuclei in young muscle cells.

out their cell bodies in a line parallel with the main axis of the fish's body. They continue this lengthening until they reach from one end of the myotome well into the mass of cells toward the other end. Two other features accompany this lengthening process; the cell becomes much larger in bulk and its nucleus changes much in character, becoming larger and oval, while its nucleoli enlarge. The staining reaction of the nucleus is very different at this time: the chromatin is spread out in more definite masses and the nucleolus loses its affinity for iron hæmatoxylin. It will still stain with it, but in the decolorizer it loses the intense black color long before the nucleoli of the undifferentiated cells do (see Fig. 86). It should be noticed here that the tissue represented in Figure 87 is more deeply stained (*i.e.* less decolorized) than that shown in Figure 86.

Immediately that this change has set in, it will be seen that the nuclei begin to rapidly divide. But not mitotically, as before. They perform a perfect amitotic division that greatly increases the number of nuclei without dividing the cell body. This latter grows longer until it reaches from the anterior to the posterior boundary of the myotome, while its numerous nuclei are stretched in a single row from one end to the other (Fig. 87). It is worth noticing that the oldest cells are always on the outer edge or layer of the myotome and hold that position through life. We find the outer muscle cells or sarcoblasts beginning to perform the next step in development before the innermost ones have changed from mononucleated, embryonic cells into the elongating and multinucleated sarcoblasts.

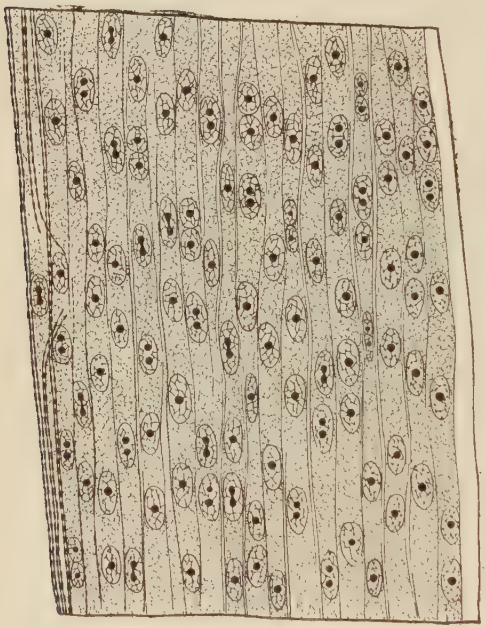

FIG. 87. — A later stage than Fig. 86 in the development of muscle tissue in the sucker, *Catostomus*. Fibrils beginning to form in the outer cells. No connective tissue has, as yet, moved in between the muscle cells. Abundant amitotic division of the nuclei.

This next step consists of the formation of the striated muscle fibrils in the cytoplasm of the sarcoblast (Fig. 87). The fibrils appear gradually and are faint at first, slowly growing more distinct as they develop. The dark anisotropic portions are to be seen first, and, from the length and arrangement of these segments, as compared with those of fully developed muscle, it seems that they are laid down and appear as fully extended fibrils from the very first. They must, of course, contract with the rest of the muscle, although, being nearest the center of the body in their weakest stages, they probably do not have to exert the contracting force to the degree that the fibrils outside of them do. It should be noticed here that the sarcoblast can contract before the formation of fibrils and that they are earliest mature, and first provided with fibrils that enable them to contract more strongly, on the outer edge of the body, where the strength can be used to greatest advantage (Figs. 86 and 87). The fibrils appear always in one side, the inner side of the cell. The nuclei are, in

consequence, pushed out to the outer edge, which position they occupy for a long time (Fig. 88).

The fibrils are laid down one after another, an outer cell always having one or two more fibrils than a cell of equal size just inside from it. This accumulation of fibrils is continued until each fiber seems to be a mass of fibrils with a little sarcoplasm clinging to it, rather than a cell containing a certain number of fibrils. For an example of a muscle cell that never acquires many fibrils, see the heart of an adult lobster (Fig. 92). While the fibrils are appearing one by one in the outer cells, the connective-tissue cells lying between the epithelium and the myotome begin to migrate slowly in between the myotomes where the connective-tissue septum is found in the adult. After they are well introduced into the septum, they send cells down between the sarcoblasts, or muscle cells, as we must call them now, into the myotome (Fig. 89). The blood capillaries follow this connective tissue later; whether from the inner or outer edge was not determined.

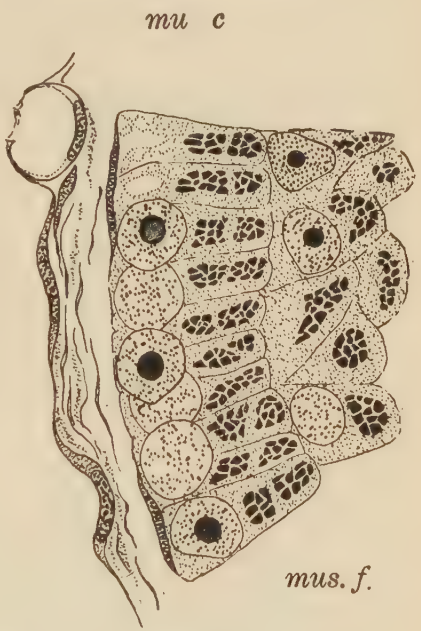

FIG. 88. — Transverse section of a bit of peripheral body muscle of the sucker, *Catostomus*. A little further developed than in Fig. 87. *mu.c.*, mucous cells of skin; *mus.f.*, muscle fibrils in groups in the proximal sarcoplasm of the muscle cells.

At this time the muscle cell is fully formed, and future changes depend only upon its growth and the addition of more muscle fibrils. It is possible that these cells or sarcoblasts divide. If so, the division is an unequal, longitudinal splitting of the cell accompanied by a proportional division of the nucleus and the muscle fibrils. Figure 82 shows, in transverse section, a possible division of this sort, where the smallest fiber seems to have recently split from the next largest. In fact the whole group of fibers, five in number, seem to have come from one original fiber.

Technic. — It is comparatively easy to secure good preparations of this tissue in its earlier stages. The tissues are soft and yield to the best fixatives in a very satisfactory manner. The staining is also easy. Flemming's fluid and two or three of the other best methods should be tried.

LITERATURE

The histogenesis of striated muscle has been described by several writers, among whom are Eycleshymer, *American Journal of Anatomy*, 1902–1903, and M. Heidenhain, *Anat. Anz.*, 1901, and J. B. MacCallum, *Johns Hopkins Hospital Bull.*, 1898.

CARDIAC MUSCLE

In considering this muscle as a class, we are departing from all former classification because several kinds of muscle included in the other classes are found in this. The group, then, is a physiological one, not founded on any common structural distinction that we can, as yet, pick out. It is usually, however, different from the other muscle tissues of the body.

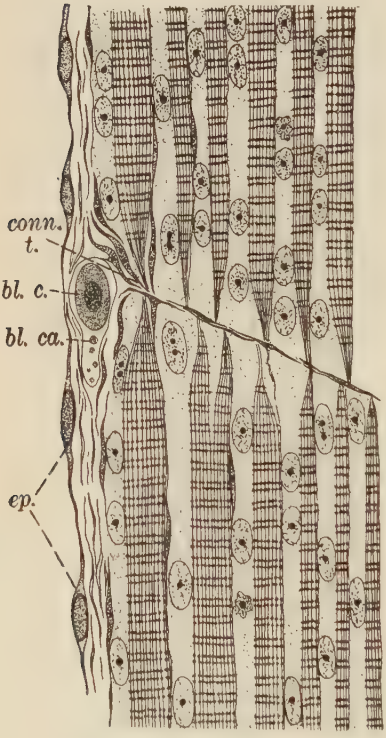

Fig. 89. — Portion of peripheral muscle tissue from the embryo of a sucker, *Catostomus*. Considerably more advanced than in Fig. 88. *ep.*, single-layered epidermis; *conn.t.*, connective-tissue cells migrating from the subcutaneous layer in between the myotomes and thence in between the muscle cells; *bl.ca.*, blood capillary containing red-blood cell and blood platelets; *bl.c.*, blood cell. From embryo of 10.5 mm.

Fig. 90. — Two muscle cells from the heart of *Unio*. Blood cell (wandering cell) attached to one. × 580.

The heart muscle is distinguished physiologically by the fact that it must keep constantly in action at a considerable rate of speed and tension. The consequent and peculiar nervous and gross arrangements are a morphological and a physiological matter rather than a histological one. Also, as the heart is elsewhere considered as a part of the circulatory channels, we shall pay attention here only to the cytology of its muscle.

A smooth muscle fiber with its nucleus and principal cytoplasm body lying outside of the myo-fibril group is described in the wall of the heart vessel of *Cerebratulus*. Other peculiar contractile cells are found in the pulsating vessels of the worms. Even in these early stages of phylogenetic development we find a cardiac muscle cell that is somewhat different from the other muscle cells of the body.

In the majority of the mollusks the heart muscle is composed of smooth cells. In *Unio* they are simple spindle-shaped forms that are

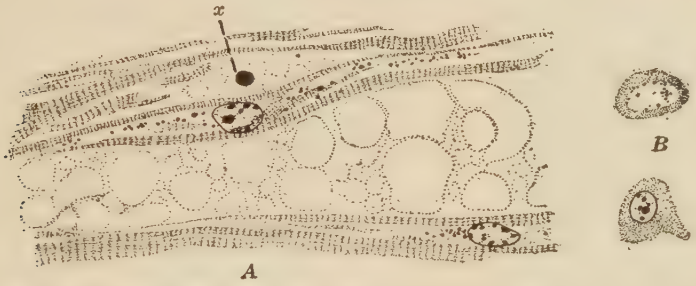

FIG. 91. — *A*, heart muscle tissue from the Gasteropod mollusk, *Sycotypus*. *B*, transections of other fibers, one through the nucleus and one a short distance from it; *x*, body of unknown function. × 700.

arranged in the peculiar mesh work which is met with in most heart muscles (Fig. 90). Besides their peculiar arrangement, they differ from the ordinary *Unio* muscle cells in having less fibrillar contractile material and a far larger, central mass of sarcoplasm. In some mollusks, especially those of highest specialization, a well-differentiated cardiac muscle is to be seen. It reaches its highest development in the cephalopods, but the relations are more easily demonstrated in a gasteropod, so we shall study the **heart tissue of** *Sycotypus canaliculus* (Fig. 91).

But little explanation is necessary. This tissue consists of cells that are spindle-shaped and contain the nucleus in the center of the fiber. Like the heart fibers of *Unio*, they also have developed the myo-fibrils in the peripheral layer of the sarcoplasm. The prominent difference is, that the myo-fibrils are striated by alternate areas of isotropic and anisotropic substance. They also have more of the fibrils developed, giving the cells a heavier and more substantial appearance.

Striation is evidently a feature that belongs to no particular set of muscle cells but may appear in any of them. This view could not be held by studying the mammalian body alone. The striation is sometimes hard to find in *Sycotypus*. One may examine many differently prepared sections and see no signs of it until, at last, the right part of the right one will show it clearly and indubitably. The striations are more easily demonstrated in the squid's heart.

Many undoubtedly smooth fibers have their fibrils lying in a waved position that simulates striation. This is the more easily seen because the angles of such waves hold quantities of the stain in a crude physical way, especially iron hæmatoxylin. In this connection we shall mention the unique case of the muscle cell, described by Schaper from the salamander's mesentery, which showed alternate light and dark bands. (As Schaper's description deals with the individual myo-fibrils, however, we must accept it as a remarkable exception.)

FIG. 92. — Transection of a single muscle fiber in heart of the lobster. Shows two nuclei, about twelve fibril bundles and four of the *x* bodies of unknown meaning. One of these lies in close contact with a nucleus; *f* marks two of the fibril bundles; *conn.t.n.*, connective-tissue nucleus or sarcolemma nucleus.

In the Crustacea and Insecta the differentiation of cardiac muscle is more complete than in any lower forms. In the insect it is precisely the same as the other body muscles, except the unimportant difference of having slightly smaller fibers and slightly narrower striations. This is pictured in Figure 141.

In the lobster we find a case that is much like that of the vertebrates, an apparent syncytium, in which striated myo-fibrils are developed (Fig. 92). Its embryology would probably show a multicellular origin, but as this is not known, we shall study the adult form.

Any section shows a great wealth of fibers running in all possible directions. This apparent lack of aim in placing cardiac fibers in any particular direction has been mentioned previously and has its mechanical advantages. The fibers are not individual so far as boundaries can be detected, but form strands of one large syncytium.

The sarcoplasm is very abundant in proportion to the other contents present and contains three prominent objects, nuclei, myo-fibrils, and characteristic bodies of doubtful function.

The *nuclei* are fine and large, as are most of those in the lobster's tissues. Like other lobster nuclei, they have a delicate but rigid nuclear membrane, finely distributed chromatin, and a small but very clearly defined nucleolus. In a transection of almost any single connecting strand there are to be seen from one to three nuclei, more rarely none or more than three. Figure 92 shows a typical transection.

The *myo fibrils* are especially worth study in such a section as the above because they are comparatively few in number for such a well-differentiated muscle cell and serve to do away with the idea, so prevalent among beginners who study only human histology, that a muscle fiber

is a series of bundles of myo-fibrils in which some sarcoplasm and nuclei have accidentally become entangled rather than a cell with all its normal organs, that contains in addition some myo-fibrils.

Our illustration (Fig. 92) shows a large developed cell that contains about fifteen small bundles of the fibrils. These bundles are subdivided into smaller bundles and the subdivisions contain on a very rough estimate about thirty fibrils on an average. The total cross-section area of the bundles would not be a third of that of the whole cell.

The sarcoplasm, like that of most muscle cells, is granular. But scattered at frequent intervals through the cell are peculiar chromatic bodies, spike-shaped, with the blunt end about as large as a cardiac nucleolus. Each one, where it lies in the sarcoplasm, is surrounded by a clear zone that is visible in the figure. Figure 92 shows four of these bodies, two seen from above and two seen from the side. One of these latter lies very close to a nucleus. The similar bodies in the *Sycotypus* heart muscle were round instead of spike-shaped.

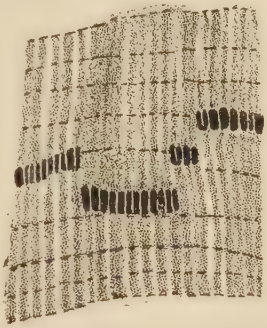

FIG. 93. — Several muscle cells from the human heart showing the "intercalated disks" where the bodies touch each other. Nuclei not shown. (After M. HEIDENHAIN. From STOHR'S "Text-book of Histology" by LEWIS.)

The fiber is everywhere covered by a clearly marked sarcolemma, a connective-tissue sheath that is made of cells, and consequently has its own nuclei.

A lateral view of one of these fiber portions will show several important features. The fibril bundles are practically continuous, running from one mesh to another of the reticulum, sometimes dividing and merging their fibrils with other bundles.

The fibrils are beautifully striated, and although it is sometimes hard to see the striations, they can be seen in many stages of contraction. The pattern of striation is the same as that of the other lobster muscle, except that the segments are much shorter, and the striations consequently much finer and more closely set. The peculiar cells found among the muscle fibers are possibly excretory in function.

The vertebrate heart, as exemplified by the heart of man, probably shows the most highly specialized cardiac muscle tissue, unless that of the insects can be so considered on account of its very perfect muscle fibers.

The human cardiac muscle is called a syncytium, although it originates as a mass of mesenchymal cells with their bodies irregularly in contact so as to form a thick-meshed reticulum. In common with the lobster's heart, its striated fibrils run continuously through the reticulum, regardless of cell boundaries.

And yet in the human tissue this continuity of the fibrils is only functional, the fibrils being broken at the cell boundaries by the interposition of small (probably non-contractile) intercalated disks (Fig. 93). We must remember that where two cells, provided with fibrils developed to sustain a strain, join each other in the line of that strain, the fibrils of each must join with those of the other, or they will not be able to perform their function. They would then soon atrophy from disuse. Read the discussion of the lobster's ligament tissue for its bearing on the fibrillar continuity of joining cells (see Fig. 67 and description).

Each fibril, in the distinct individual cells of the mammalian heart muscle, joins the fibrils of an adjoining cell in order to have a strong and functional point of attachment. It is very doubtful if this muscle cell supports any but its own fibrils trophically, or even furnishes them a nervous stimulus, although this latter case is more probable than the first.

Each cell is possessed of a single large nucleus, as a rule, although there may be two. In many cases the appearance leads one to think that an amitotic division is taking place, which is not probable. The cells are branched at an acute angle, and by joining their short processes with the other muscle cells they form the muscular reticulum. The cell boundaries are sharply marked by the rows of "intercalated disks," which so resemble one of the striations that unless stained specially they are not easily seen. A connective-tissue sarcolemma invests all parts of the cardiac fiber reticulum. Its narrow, dark nuclei form a sharp contrast to the full-bodied oval nuclei.

The striation of this muscle bears the same relation to the body muscle in man that the two bear in the lobster; it is the same in structure but much finer. Because of this it is sometimes a little hard to demonstrate.

About the only two generalizations that we can extract concerning the cardiac muscles are: first, the cells form an irregular reticulum which can be explained on mechanical grounds; and secondly, the striation or segmentation of the fibrils is finer than that of the other body muscles. The latter feature probably has some unknown physiological significance.

Technic. — The technic of cardiac muscle hardly differs from that of ordinary striated muscle. The tissue is a little more apt to become brittle and a little more difficult to stain. The intercalated disks are brought out by the use of nitrate of silver.

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SMOOTH MUSCLE TISSUES

Smooth muscle originates in the embryo as a specialization of mesenchymal cells. It appears as a set of unicellular fibers, all lying parallel for mutual support in their efforts. Sometimes they are crossed or interlaced, but in this case only certain sets act together to effect certain motion. All the fibers in one direction may contract to cause a shortening of some part of the body; again, all the fibers lying in several directions in one plane or in two given planes may contract to force an extension of a part of the body in another direction or plane. In form, these fibers are almost always elongated spindles tapering into thin ends. Very rarely these ends may be branched into two or three main branches, as is sometimes found in the young mammalian aorta, or they may give off many fine side branches, as in the smooth muscle cells of the heart of some mollusks. These side branches are not contractile, however.

The smooth muscle fiber is always formed in a single cell and has a single nucleus. We recall no case where it has the syncytial multiplicity of nuclei found in the large striated muscle cells. This nucleus may appear inside of the fiber or outside of it. This idea may be as well expressed by saying that the myo-fibrils are distributed around the nucleus or to one side of it.

The smooth muscle cell, like all muscle cells, owes its contractile power to the development of a varying number of myo-fibrils in its cytoplasm. These fibrils may be very few and appear unimportant in the make-up of the cell, or they may be many, and at first sight be all of it. Perhaps the most ordinary method of their appearance is in the entire periphery of the cell where they form a thin layer (see Fig. 97), or a thick layer that occupies most of the room in the cell body (see Fig. 99). When the fibrils appear in bundles or a single bundle in one side of the cell-body, their development, if large, leaves the cell body as an apparent appendage on one side. This is seen in some low forms, especially well in the nematode worms (see Fig. 95).

The smooth muscle fiber is generally used by animals that move slowly or in parts of the anatomy of more active animals where a slower motion not directly controlled by the will is needed. There are a few examples, however, of organisms which are noted for their swiftness and beautiful muscular activity, and yet have nothing but smooth muscle to perform their actions with. The squid is such an example, and its muscle is also absolutely under the control of the larger and central nerve centers, making it "voluntary" in a proper sense.

A contractile fiber from *Euspongia officinalis* will show a very low form of smooth fiber with very few fibrils and much granular sarcoplasm.

This structure has but weak powers of contraction and might be considered as half connective tissue rather than muscle (Fig. 94).

Cerebratulus **shows an interesting form of fiber** in which the fibrils

FIG. 94. — Two contractile fibers from *Euspongia*. (From SCHNEIDER after F. E. SCHULTZE.)

are few and in another part of the cell from the nucleus. See the description of the blood vessels in this form for descriptions and illustration (Chapter XII).

The longitudinal muscle fibers of *Ascaris megalocephala* show a very highly specialized cell in which the nucleated cell body is situated on the inner side of its plate-shaped muscle bundles. The bundles consist of a single row of fibers embedded in a cement substance of considerable thickness. The cell body sends off some peculiar processes, one of which is said to have a nervous function. Figure 95 was drawn from the common *Ascaris* in the cat.

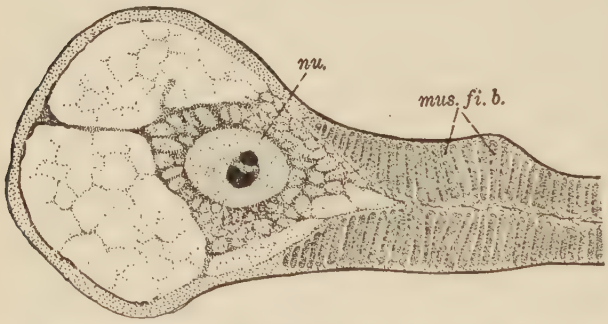

FIG. 95. — Transection of body-wall muscle cell of an *Ascaris* from the cat's pyloris ; *nu.*, nucleus ; *mus. fi. b.*, muscle fiber-bundles in transverse section. × 1000.

The gizzard of the earthworm is provided with a covering of very well-developed and closely packed fibers of rather short length. Their sides are flattened from close contact with their fellows and the edges are sharp. The outer edge is flat and the inner edge sharp, the whole effect being that of a forged knife blade at each end. One end of such a fiber is seen in Figure 96.

The body of the fiber is composed of a very coarsely granular sarcoplasm, containing a great many thick myo-fibrils that run in slightly curving lines from end to end. The fibrils are thickest near the surfaces, and only in the interior of the fiber is it possible to see the sarcoplasm. It occurs here in several long, spindle-shaped masses, in the largest of which lies the nucleus. This nucleus is oval and not specialized in any particular way. It is not even much elongated, as many such nuclei would be under similar conditions.

A sarcolemma has not been demonstrated. Its presence in this muscle would demonstrate the presence of a real sarcolemma on a muscle cell on account of the small amount of connective tissue in the organs of the earthworm. A notable feature of this cell is the series of fine fibrils that are given off from its body to connect it with the other similar muscle cells with which it is in contact. These connections must

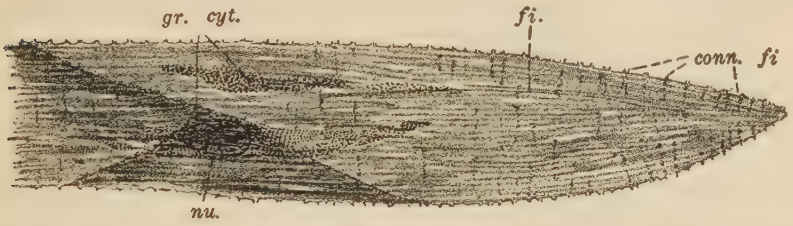

FIG. 96. — End of a single muscle fiber from the gizzard of *Allolobophora*. Teased preparation. *gr.cyt.*, granular cytoplasm; *nu.*, nucleus; *conn. fi.*, connecting fibrils broken by testing; *fi.*, muscle fibrils. × 435.

be of considerable strength, as the muscle could not hold together and operate if they were not.

These intercellular connecting fibrils are a part and product of the muscle cell, and we may say that this cell forms and uses both myo-fibrils and connective-tissue fibrils. They are placed in short rows and appear to emerge from the body of the cell between the myo-fibrils. Seen with a lower power, they lead one to think that the muscle cell is a striated one. They are broken off a short distance from the surface of a macerated fiber where the neighboring fiber was torn away. Their presence, under the high power, gives the whole cell a rough or prickly appearance.

Such structures do not represent any vital connection between the cells, not being protoplasmic in nature. They cannot, therefore, be called "protoplasmic bridges," and should not be called intercellular bridges, as that has come to mean the same thing.

The muscle fibers of the squid should be briefly compared with the one we have just examined. This muscle cell is of extreme length and is attached, where it meets with a surface, by a blunt end; or where it is interlaced with other fibers, by a long, thin ending. Its myo-fibrils are massed on the surface of the fiber in a thin layer, thus leaving a large cavity in which the watery sarcoplasm lies.

The nucleus occupies the central part of this cavity, and is enormous compared with the nucleus of the earthworm's gizzard cell. It makes a beautiful object for the study of the nuclear organs. Figure 97 shows several transverse sections and a longitudinal section of such fibers in the squid's arm.

The powerful closing muscles of the plecypod mollusks show a fiber

that is unicellular and spindle-shaped, and has all the characteristics of smooth muscle except one (Fig. 98). When contracted, it shows, not a transverse striation, but a peculiar series of oblique striations, little groups of which pass each other at an angle. The exact nature of this striation

FIG. 97. — *A*, longitudinal sections of several muscle fibers from the squid, *Loligo; B*, transverse sections of the same at different levels. × 700.

has not been determined upon, and so it cannot be compared with that of ordinary striated muscle. So, also, the cell itself cannot be accurately compared with other unicellular and spindle-formed muscle cells. Both this last form and that of the squid are "voluntary fibers."

FIG. 98. — Portions of two longitudinal sections of fibers from the closing muscle of *Venus*. *u.*, nucleus. × 870.

The involuntary muscle cell of the vertebrates will form our next and last example of this class of muscle fiber. This resembles the squid's fiber in that its myo-fibrils are laid down in the peripheral sarcoplasm. It shows more of them, however, so many, that but little sarcoplasm can

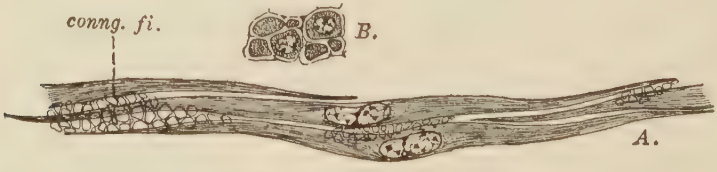

FIG. 99. — *A*, longitudinal section of several smooth muscle fibers in the bladder of a calf; *connng. fi.*, connecting fibrils between the cells; *B*, transverse section of similar fibers.

be found. What can be seen lies at either end of the rather elongate nucleus. This cell has its own variations in the different forms of vertebrates, and our example is taken from a section of the bladder of a calf (Fig. 99). In some other forms the nucleus is much longer and thinner, and can be seen to twist and curl when the muscle contracts. The

largest of these fibers is seen in the pregnant uterus of mammals at term, when the fibers attain an enormous size.

The smooth muscle cells in the walls of the calf's bladder show an intercellular fibrous connective tissue. This can be differentiated from the muscle substance by staining, and it appears as a fibrillar material in our figure. It is produced, as was the similar substance of the earthworm's gizzard cell, by the muscle cell.

A section of any tubular portion of the digestive tract of a mammal embryo of the right age will show the origin of smooth muscle. Lewis has described the process in the esophagus of a pig of 8 mm. (Fig. 100). The organ consists at first of an inner stratified epithelium resting on a mesenchymal layer of primitive connective tissue whose cells have not yet begun to form the fibrils. When the fibrils do appear, they are myo-fibrils, all lying in the same direction and filling the cells up until they are the familiar smooth muscle cells.

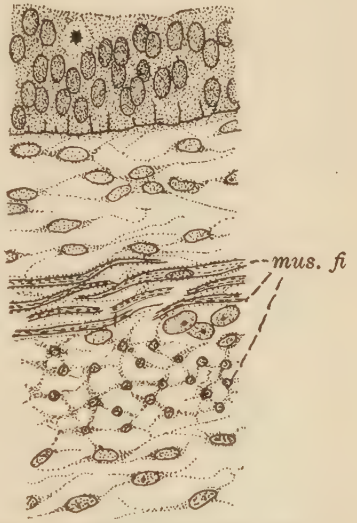

Fig. 100. — Part of a transverse section of the digestive tract of an embryonic pig. *mus.fi.*, developing smooth muscle fibers. (From "Stohr's Histology," by Lewis.)

These cells do not appear throughout the mesenchyme, but in two layers of it, the inner of which produces fibers that run in a circular direction, while the outer one lays them down at right angles to these and parallel to the axis of the tube. Both these layers are removed from the epithelium by a third, which remains a true connective tissue, becoming a layer of soft, fibrous connective tissue in the adult state. Even while forming the muscle fibers, the cells remain partly connected by processes which, at this stage, are true intercellular processes. This clearly appears in the transections of the longitudinal or outer layer of muscle cells, and these bridges form the fine intercellular connective tissue, afterwards retreating into the cell and becoming part of its sarcoplasm.

Technic. — Smooth muscle differs from the other kinds only in the way that several special processes can be applied to it. Its cells are most easily isolated by maceration and teasing, even after they have been fixed in several fluids. Such preparations should be supplemented by well-stained and thin sections in which the finer cytological details can alone be brought out. There are stains that are specific for the smooth muscle fiber when there is doubt as to whether it is muscle or a connective-tissue structure (see Lee).

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UNUSUAL FORMS OF MUSCLE

Muscle, as has been said, must be developed wherever needed. Having accounted for some of the usual places and methods of formation, we find a few very unusual forms which cannot be brought under the other classifications.

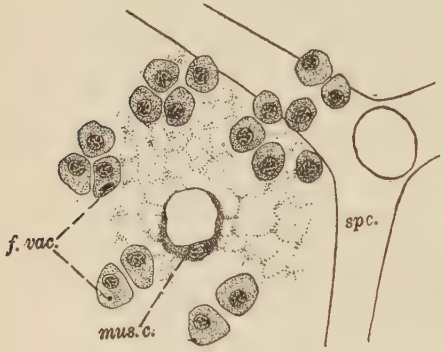

FIG. 101.—Part of the body wall of a simple sponge, *Leucosolenia*. *f.vac.*, food vacuoles in endodermal cells; *mus.c.*, muscle cells surrounding the water pore (*p.*); *spc.*, spicule. × 900.

An example of such a peculiar muscle cell is to be seen in the large epithelial cell that lies next to the water pore in a sponge, *Leucosolenia* (Fig. 101). The edge of this cell is transformed into a fiber-bundle that surrounds the pore, and, by contracting or relaxing, it regulates the flow of water. The outline of this cell is not to be seen, but is undoubtedly a definite outline and perhaps a regular one, like most epithelial cells. It could be brought out, perhaps, by the use of silver nitrate

Another muscle is peculiar from the remarkable way in which its cell-body is separated from the myo-fibrils. This is seen in **the muscle of the parasitic *Cercaria* from *Helix***. The cell-body of this muscle cell lies entirely apart from the fibrils and sends to each one a single strand of cytoplasm to support it (Fig. 102). It is probable that each strand covers all of the fibrils that it goes to, on account of the needs of trophic and functional support. A fibril cannot act alone if any of our conceptions of muscle activity be near the truth. The fibril must have some portion of sarcoplasm in contact with it to furnish it (according to Englemann's theory) with the requisite heat-oxydization that causes it to swell and shorten.

The stalks of some Protozoa, as *Vorticella*, are capable of a very strong and rapid contraction (Fig. 103). This seems again to be a case where protoplasm cannot contract with sufficient efficiency, but yet is capable of developing an arrangement which can so contract for it. The fibril

in the stalk is a product of the thin layer of cytoplasm that surrounds and nourishes it.

The fibril is not like the ordinary muscle-fibril in shape and mode of action. It is very large and heavy, and when it contracts it does so by contracting one of its sides. As, according to Entz, this side forms a

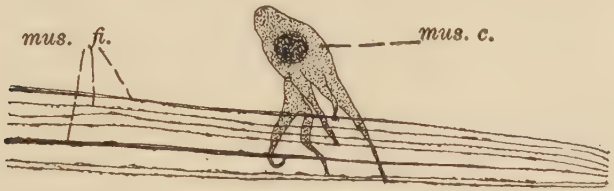

FIG. 102. — Muscle cell of *Cercaria* from *Helix*. *mus.c.*, muscle cell; *mus.fi.*, muscle fibrils. (After BETTENDORE.)

spiral band around the fibril, the result is that the fibril is thrown into a spiral shape resembling a wire bed spring. Our specimens, and consequently the illustration, did not show this spiral band.

The cilia and flagella found on many cells all through the animal

FIG. 103. — Proximal portions of two *Vorticella* showing the insertion and upper parts of the contractile stalks. × 800.

series, are one of the most primitive forms of motion that affect the cell's relations with the exterior (see other figures).

These organs are probably passive rods of various lengths and thicknesses which are moved by the cytoplasm in or near the edge of the cell. They are, apparently, continuations of fibril-like areas inside the cell. This last connection has been used to liken the cell fibrils of the cilia to the rays of the centrosome, which are also organs of motion, and to come back to the centrosome as the fundamental cell-organ of motion.

While attractive, this hypothesis is not grounded upon sufficient knowledge and should not be too seriously entertained.

Technic. — The sponge material had best be fixed in pure watery solution of sublimate and, after the mercury compounds have been taken out with potassium iodide, stained with weak alum carmine progressively, that is, stained slowly with a very weak solution of the stain, so that a subsequent decolorization is not necessary.

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CHAPTER IX

ELECTRIC TISSUES

In a very few organisms, certain tissues are able to produce electricity. They are especially developed and constructed to do this, and they produce it specifically, and apart from the electricity generated in small quantities as a by-product in some other tissues. These few animals are all fishes, some teleosts, some elasmobranchs.

Electric tissue is composed, in the few known cases where it occurs, of a series of plate-like units, each of which may be designated by the name *electroplax*. Each electroplax lies in a connective-tissue compartment, imbedded in a jelly-like mass of tissue which fills the compartment. The nerve and blood supply come from some side or corner of the compartment and are distributed through the jelly tissue to the electroplax. These plate-like electroplaxes are arranged in rows or are irregularly massed. All the electroplaxes in a given fish are oriented alike.

The electroplax may be considered to be a single cell with many nuclei or, in other cases, as a syncytium formed by the union of a number of cells. Some might consider it an organ unit composed of many cells on account of the fact that each nucleus is surrounded by its own portion of unspecialized cytoplasm, but it can probably be considered better as a syncytium in the same sense that a voluntary muscle fiber is so considered.

The electroplax is composed of three principal layers, a *nervous* or *electric layer* forming one surface on which the nerve ends, a *middle layer* which may be called the *striated layer*, and a *posterior layer* whose exact function is not known, but may, perhaps, be a *nutritive layer*. This layer is really a part of the anterior or electric layer, and is continuous with it around the edge of the middle layer. In fact, the nerve sometimes passes through or around the entire electroplax, and turns, to branch out and innervate the posterior layer instead of the anterior (*Mormyrus*). The apparently different functions of these two similar layers are then reversed. The striated layer is sometimes missing.

These three layers form the body of the electroplax, and this body is best understood by comparing it to a voluntary muscle fiber which has grown wider and shorter until it is wider than it is long. This process is carried on to different degrees; only slightly in some rays, more in others,

and in *Mormyrus* it is well developed; while in *Torpedo* it is carried to such a degree that the electroplaxes are wide and thin; so thin that layers can hardly be discerned. The broadening and development of the electroplax takes place at one end of the changing muscle fiber in both the ontogeny and taxonomy of the electric tissues, and sometimes the lower end of the electroblast or young electroplax remains attached to the posterior surface. Figure 104 represents a series of diagrams of

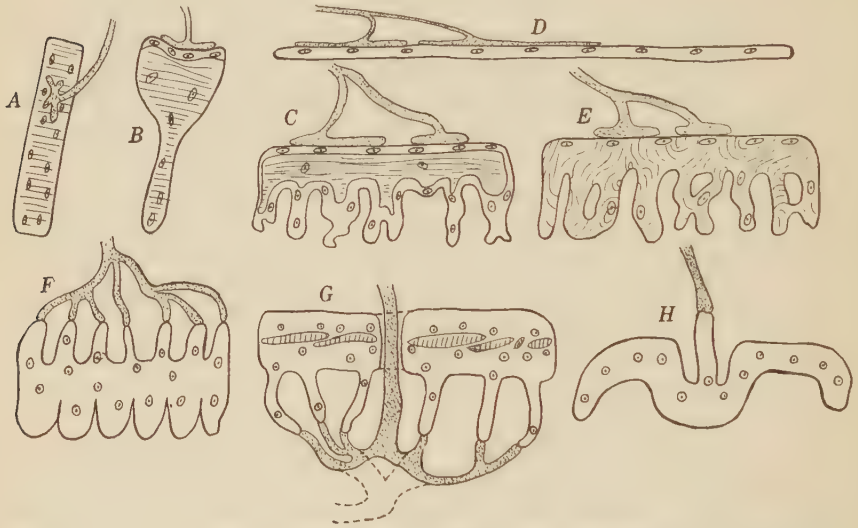

FIG. 104.—*A–H*. A series of diagrams of the various kinds of electroplaxes, showing in part their polarity and their relations to the striated muscle fiber. All nerve structures stippled. Striated structures indicated by lines. *A*, muscle fiber; *B*, electroplax of young *Raja batis; C*, electroplax of *Raja lævis; D*, electroplax of *Tetronarce; E*, electroplax of *Astroscopus* the "stargazer"; *F*, electroplax of *Gymnotus*, the electric "eel"; *G*, electroplax of *Mormyrus; H*, electroplax of *Malapterurus*. Innervated surface above, on all but *G*.

the various forms of electroplaxes, showing their polarity and comparing them with a striated muscle fiber.

The electroplax is developed, with the possible exception of *Malapterurus*, from an *electroblast* which is exactly homologous with a sarcoblast or young muscle cell, and in some species of *Raja* are the same, as far as the microscope can reveal. Thus we see that the electroplax may be considered to be a modified muscle fiber whose action produces electricity instead of motion.

The distribution of nuclei in the electroplax is characteristic; they form a continuous single layer in the electric layer and posterior layer and are separated by regular intervals from each other.

Each is surrounded by a bit of granular, undifferentiated cytoplasm, much as is the nucleus of a smooth muscle cell in the vertebrate bladder

or intestine. Others are found scattered sparingly through the middle layer. The middle or striated layer represents the striated body of the striated muscle fiber in which the prominent lamellæ of muscle units are more or less absent. In some of the most powerful and efficient electric organs this middle layer is apparently missing, leaving only parts, the electric network, which exists between the lamellæ in less specialized forms and also may exist in normal striated muscle cells. A thin membrane surrounds the whole syncytium and is called the *electrolemma*.

The specific cell-organ through the action of which electricity is discharged is probably a series of fine rod-like structures attached to the electrolemma and directed towards the anterior layer on which they rest. They have not been actually described as present in all electric tissues, but are probably so present. They are exceedingly small and in some forms are straight and simple, and in others are curved and provided with peculiar end-knobs, and sometimes are combined into groups of two or three.

These *electric rods* are sometimes found all over the surface of the electrolemma; in other cases they occur only at, or near, such points of it as are touched by the end-plates of the nerve that supplies it.

While the electric rods are probably the means through which the electricity is discharged, there should be another cell-organ by which it is stored up in some potential form through nutritive processes. A protoplasmic network of fine, irregular fibers in which granules are embedded has been surmised, in *Raja* and other forms, to be such an organ. This network pervades the entire electroplax more or less, being found between the lamellæ only of the striated layer when these lamellæ are present. This network probably acts as an area of deposit for the numerous granules that are secreted by the cytoplasm. We shall call such granules the *electrochondria*, as they are probably homologous to the myochondria of the muscle cell, and used to produce electricity by some chemical process.

The nerve supply enters the compartment, usually from an anterior corner, as one or more medullated fibers derived from the electric nerve, which is a modified motor nerve. These fibers lose their medullary sheath soon after entering the compartment, and branch and rebranch until they touch the electroplax at many points and spread out into the end-plates. These nerve end-plates form an irregular but characteristic network that covers most of the electric surface of the electroplax. This area of contact between nerve and electroplax is reduced to a number of evaginated points in *Gymnotus*, the electric eel; to still fewer in *Mormyrus*, and to one point in *Malapterurus*, the electric catfish.

The rays, *Raja*, present an electroplax in which the general features

of structure can probably be demonstrated and their relations understood as well as in any form. The electroplax is not highly specialized, and material is easily obtained all over the world. A complete demonstration is somewhat delicate and difficult, and nitrate of silver preparations are essential to a demonstration of the fundamental points.

Technic. — It is more true of electric tissue than of muscle that chance plays a large part in the winning of good results. Several of the better fixatives should be tried, and the use of dead tissue which has recently died of secondary causes (as bleeding or suffocation or narcotics) should not be neglected. Nitrate of silver used after the rapid method of Golgi is an all-important method in the study of electric tissue, and should be used in the study of each form. It serves to demonstrate much more than the nerve connection of the electroplax.

LITERATURE

No really comprehensive survey of the subject has been written other than the shorter accounts in the text-books of zoölogy and some encyclopædias. The subject must be read up in the separate articles, some of which are mentioned after the following parts.

ELECTRIC TISSUE OF ELASMOBRANCH FISHES

The electric tissues of *Raja ocellata* consist of two modifie regions of the tail muscle; a symmetrical spindle-shaped area in each of the muscular masses that lie, one on each side of the tail. The spindles begin anteriorly in this ray at about the level of the ventral fins and extend almost to the end of the tail. The electric tissue can be easily distinguished from the surrounding muscle tissue in the fresh specimen by its jelly-like appearance.

The organ is divided into minute compartments whose outline is apparent on the outer surfaces as well as on cut surfaces of the spindle. The dividing walls of these compartments are of a white fibrous connective tissue, and the interior of each compartment is filled with a jelly-like connective tissue, the *electric connective tissue* in which the *electroplax* lies. From the anterior, inner corner or edge of the compartment, a nerve supply enters to innervate the electroplax. Blood vessels are introduced, usually from the opposite or posterior side, and branch in the electric connective tissue to furnish the electroplax with blood.

The compartment is wider than it is long, a polyhedral cavity placed with its two large surfaces cephalad and caudad. It will average, in a three-foot *ocellata*, about 600 to 800 microns in width and 390 to 450 microns in length. The myotomes of the tail muscles are continued directly into the electric organ, dividing its mass of electroplaxes into a

series of concentric cones that may be called the *electrotomes*. The relations of these electrotomes to the myotomes are further explained in the next section on the development of the electric organs in the embryo skate.

The *electroplax*, which is the structure that produces the electricity, is a large, disk-shaped syncytium that lies within the compartment with its width agreeing with the width of the cavity. It does not occupy the entire length of the cavity, however, but leaves an anterior and a posterior space. These spaces are usually of considerable size and, as has been indicated before, are filled with the electric connective tissue whose individual cells are branching forms, as seen in Figure 105. These cells secrete the jelly-substance of the tissue.

FIG. 105. — Portion of an electroplax of *Raja lævis*. *el.c.t.*, electric connective tissue before the electroplax; *el.n.e.*, electric nerve ending; *el.l.*, electric layer; *el.nu.*, electric nucleus; *str.*, striated region of electroplax; *nut.l.*, nutritive layer. The mass of striated material in the large central papilla is cut off optically from the striated layer by the irregularity of the papilla.

The spaces vary much in different skates, the anterior being many times larger than the posterior in a *Raja ocellata*, while in *Raja lævis* the exact opposite is true. It is in the electric connective tissue of the anterior space that the nerves supplying the electroplax ramify. These nerves, which consist of several medullated fibers, when well within the compartment, lose their medullary sheaths and the fibers begin to divide and sub-divide as they approach the anterior surface of the electroplax on which their ramifications terminate in a large number of end-plates.

These end-plates appear, in a section, as a rather closely set row of tiny, transparent areas lying on the anterior surface of the electroplax (Fig. 105, *el.n.e.*). The finer non-medullated ramifications of the nerve are closely associated with the connective-tissue cells whose nuclei are to be seen lying alongside of the branches of the fiber at many points near the electroplax (Fig. 105, *el.c.t.*).

The shape of the electroplax is that of a rather thick disk with its circular edge slightly thinned and bent upward (anteriorly). It has, therefore, been called saucer-shaped or, where in other skates the bent edge is higher, cup-shaped. Its shape varies much in the different species of skates.

The electroplax is composed of three layers, the two outer of which are continuous around the edge and must be regarded as two (an anterior and a posterior) specialized areas of a general outer layer. The middle or inner layer is of a very different structure and much the thickest. It forms a core or inner portion and is striated. These striations represent cross sections of a series of undulating and parallel lamellæ. They correspond to similar structures in striated muscle, and under the highest powers are seen to be formed of sheets of upright rods.

With different fixatives and in different specimens the appearance of these rods varies, much as does that of the muscle-rods. At times a row of dots forms an equidistant line between them and again the rods themselves appear double or very short, with two dots on either end. A fine transparent fibril can be seen running at right angles to the lamellæ and connecting the rods into fibrillar structures like those of muscle. This fibril probably is homologous with that of striated muscle tissue, and the striation is due to the exact and equal arrangement of the anisotropic and isotropic substances on the parallel fibrils. Their very small size prevents satisfactory studies of their structure.

The lamellæ are very much denser and finer in *Raja lævis*, and it is, in this latter species, almost impossible to see the rods that compose an anisotropic line. Here, too, there is far greater density and continuity of the striation, and they are not so irregularly arranged as in *ocellata*. The whole syncytium is surrounded by a delicate cell-membrane, the electrolemma, which corresponds to the sarcolemma in a voluntary muscle cell.

While the anterior surface is flat the posterior surface is evaginated into many papillæ that vary in the individual electroplaxes as to width and length. The posterior layer covers these papillæ and follows all their turns and bends. Where the width of a papilla is less than that of a double thickness of the posterior layer, no striated or middle layer exists except certain broken off and irregular portions of striated material lying in the papillæ. In *Raja lævis* the striated substance is pushed

farther down into the papillæ, and larger areas are isolated from these main masses.

The nuclei are found in all three layers, forming a very regular, close-set arrangement in the electric layer, very sparingly scattered through the striated region, and numerous, but irregularly arranged, in the nutritive layer. The nuclei of this last region seem somewhat smaller, more irregular in outline, and with denser chromatin masses than those found in the other layers. Each is surrounded by a mass of granular cytoplasm. These masses are connected and form a separate layer.

The above section that we have been studying is a longitudinal, vertical section of the spindle, and consequently a longitudinal section of the electroplax. The material was fixed in Flemming's strong fixative and stained in iron-hæmatoxylin. In order to proceed farther with an understanding of the tissue it will be necessary to prepare other fresh material with Golgi's quick silver method and to cut sections at right angles to the anterio-posterior axis of the electroplax. The sections will be parallel to the neuro-electric surface, the flat surface on which the nerve terminates in many fine branches. When such sections are examined as include all or any portion of this surface, we can distinguish, in favorably stained parts of the specimen, the following facts: —

The ground substance of the electric layer forms a coarse network, whose regular rounded meshes contain the nuclei. Each nucleus is surrounded by its own portion of unspecialized cytoplasm, and a thin layer of the ground substance covers the top and bottom of each mesh with a delicate layer. Thus each nucleus, with the unspecialized cytoplasm that surrounds it, is inclosed in an oval space entirely within the electric layer.

The ground-substance shows a distinct reticulum or network of fibrils. This network is very fine and dense and stains brown-black. Its fibrils are irregular and granular, and at times can be seen to run some considerable distance without anastomosing. The reticulum is coarser in some regions than in others, and this is so marked that we may distinguish between a fine and a coarse reticulum.

How far does this reticulum extend into the substance of the electroplax and what relation has it to the striations? A cross section of the silver material will show this, and in it we see that it pervades the ground substance and is found between the striations or lamellæ only. Thus it is not directly continuous in the electroplax.

A remarkable feature that is revealed by the silver process is the multitude of tiny pointed rods projecting from the inner surface of the electrolemma into the electric layer for a distance of several microns. These structures are very dense and refractive and are only found on that part

of the electric surface that is in contact with the ramifications of the nerve-ending. They are sharp, wedge-shaped, and very small (Fig. 106).

A brief examination of the electroplax of the "torpedo fish," *Tetronarce*, should be made at this point to compare its highly specialized electric unit with the simpler and more rudimentary structure of the electroplax found in *Raja*.

In *Tetronarce* the electric organ is composed of two masses of vertical columns, each column with six sides to fit closely with its neighbors. Each column is a pile of very thin electroplaxes that lie with their two surfaces at right angles to the column and occupy its entire section, excepting that their corners are somewhat rounded. A portion of electric connective tissue lies on each side of the electroplax and they are farther separated by a very thin layer of white fibrous tissue. The columns are bounded on their sides by comparatively heavy walls of the white fibrous connective tissue.

FIG. 106. — Part of the nerve-ending on an electroplax of *Raja clavata*; *nv. f.*, nerve fiber. Nerve-ending seen behind and studded with the electric rods. (After BALLOWITZ.)

Examination of a vertical section of a part of the column shows the electroplaxes in cross section. They are so thin when seen thus that it is with great difficulty that they are made to appear as more than a line with several nuclei on it. The nuclei are clearly made out to be of two kinds: a large round one on the upper side and really lying in the electroplax, which is thinner than the diameter of the nucleus; also a smaller and somewhat elongated nucleus of denser appearance, which is clearly a connective-tissue element. This is usually lying on or near the electric surface of the electroplax. A fairly well fixed specimen of this tissue shows two visible layers of the structure.

Surface studies of this tissue with the Golgi method show, as in *Raja*, the electric end-organ of the nerve supply as well as the numerous small rods that point down into the electric layer and are found on the electrolemma only at such of its points as are in contact with the nerve end-organ. This latter structure is as complicated as in the skate. Each of the rods, instead of being a simple pointed wedge as in the skate, is of stouter formation, and bears on its end a peculiar round knob. Sometimes two of these rods are united.

The Golgi method also shows a reticulum in the cytoplasm that is similar to that seen in the skate. In general, the electroplax of *Tet-*

ronarce is similar to an electroplax of *Raja* which has become wider and very much thinner, so thin that the striated layer is obliterated and the others reduced to a minimum.

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ONTOGENETIC DEVELOPMENT OF THE ELECTROPLAX IN ELASMOBRANCH FISHES

A study of the **developing electric organ in a skate** is most illuminating as to the real significance of this tissue. It has been worked out in a common form of skate, *Raja batis*, by Dr. J. C. Ewart, and the following description is drawn from that paper and the paper by Englemann.

The young embryo of this skate has no indication of any electric tissue. The place that will be occupied by this organ, a little later in the development, is filled by the young muscle fibers that differ in no visible way from those about them (Fig. 107, *A*).

In an embryo of about 7 cm. the first appearance of the development of electric tissue is a swelling of the anterior ends of the muscle fibers in the centers of the future electric spindles (Fig. 107, *B*). The nuclei have increased in number in this enlarged part of the fiber, and some of them apparently have migrated from among the muscle fibrils and come to lie in the undifferentiated cytoplasm between the fibril-bundle and the sarcolemma (by sarcolemma in this case is meant a cell-membrane which is somewhat more evident in young muscle cells and in some electroplaxes than in most older muscle fibers). These changes first occur in the fibers that occupy the central axis of the future spindle-shaped organ and then in successive outer shells of the fibers until the organ is completed. All future changes occur in the same order.

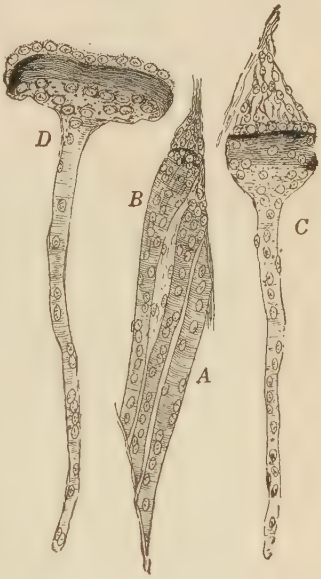

FIG. 107.—*A–D*. Four stages in the development of an electroplax from a muscle-like electroblast in *Raja batis*. *A* is in all respects like a muscle fiber; *B* shows an enlargement of the anterior end; *C* and *D* show the steps which practically complete the process. (After J. EWART.)

In an embryo of about 10 or 11 cm. in length, enough variety and advancement in the development of the electroplax can be found to supply all needed steps. The muscle fiber is seen here with the anterior enlargement much wider and heavier and showing the form and structure of the completed electroplax (Fig. 107, *C*). The posterior end, on the other hand, is arrested in its growth and development and in the older cells is actually shriveled into a ribbon-like form that still clings to the electroplax (Fig. 107, *D*). It retains its striated fibrils for a time, but they are gradually absorbed until nearly lost. This muscle-cell remnant is entirely missing in most other skates.

The further following changes have also taken place in the somewhat older stages. The motor nerve-ending of the young muscle fiber has moved to the anterior end of the developing electroplax and has developed to form the electric nerve-ending which lies at this time in a saucer-shaped depression on the end of the structure. The striated portion of the muscle fiber has widened and shortened to form the striated layer of the electroplax, meanwhile changing its comparatively wide and straight bands of anisotropic substance to narrower and curved bands. These striations of the electroplax are still, however, as strictly parallel as were the muscle striations. The changes undergone by the striation have not, as yet, been properly investigated.

The electric layer has been formed from a layer of the muscle-nuclei lying in the undifferentiated cytoplasm of the electroplax, and this electric layer has extended over the edges of the electroplax to become the nutritive layer on the posterior side. This nutritive layer has become evaginated into a number of papillæ of considerable length, extending into the electric connective tissue and anastomosing with one another to a considerable extent.

So we see, in this development, the visible and undoubted steps of the change of a muscle fiber into an electroplax; thus abundantly corroborating the surmises that this was the case in the electric tissues of *Mormyrus* and the other forms, excepting only *Malapterurus*.

The development or histogenesis of the electroplax in *Torpedo* can again be compared with the process in *Raja* to great advantage. The organ begins in this latter form as a set of vertical bundles of long thread-like cells each with one nucleus (Fig. 108, *A*). These cells acquire a faint striation as does a young muscle cell, and then the lower end begins to enlarge, and the nucleus which is near the lower end begins to divide (amitotically probably) into the large number of nuclei that are afterward found in the electroplax (Fig. 108, *B*). The club-shaped end rapidly widens into the plate-like electroplax, while the upper end of the fiber atrophies and is seen no more. The striation persists for but a short time in its ventral edge and then disappears also. The striation

seen in the latter parts of the development is the longitudinal striation or fibrillation (Fig. 108, *C*). Earlier in the process the cross striation is the most prominent. Each bundle of fibers develops into one of the columns of the completed electric organ. In changing its longitudinally placed sarcoblasts (they might more properly be called electroblasts) into electroplaxes, they are changed from the vertically elongated form into a horizontally elongated form without changing in the least their morphological position or axes. The selachian fishes thus show a complete homology in their electroplaxes. Unfortunately we do not know the embryology of the more numerous teleostean forms.

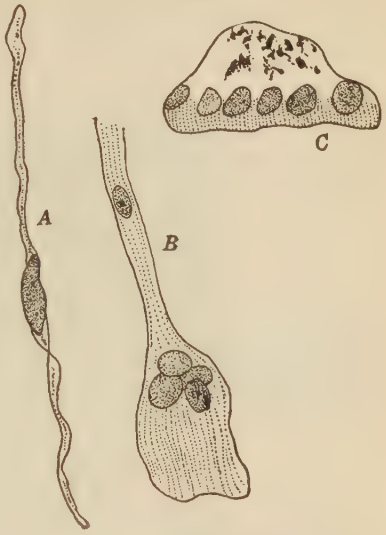

FIG. 108. — *A–C*. Three steps in the histogenesis of an electroplax of *Tetronarce*, the "torpedo" or "numbfish." The body of the electroplax is derived from the posterior end of the fiber instead of from the anterior, as in *Raja*. (After OGNEFF.) The weak striæ represent the fibrillation rather than the cross striation of muscle.

Technic.—The use of nitrate of silver by the Golgi method has been carried even into the embryonic tissue, although without the decided results that it has yielded in the adult tissue. Methylene blue has not been successfully used in any electric tissue with the exception of *Raja* by Retzius, who, however, has neglected to announce the secret of his success, if there be any secret that can be stated.

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THE ELECTROPLAX IN TELEOST FISHES

The teleost fishes present a number of examples of electric tissues which are, with one exception, recognized to be modified muscular tissue upon anatomical and histological grounds. Unfortunately the embryology and histogenesis of these organs has not been investigated for lack of material. This is especially unfortunate in the case of *Malap-*

terurus, whose electroplaxes are placed in the skin and are consequently in doubt as to their origin.

We shall briefly describe the electroplax as found in three genera of teleost fishes, *Gymnotus*, *Mormyrus*, and *Astroscopus*, making some comparisons and afterward discussing briefly the peculiar electric organ of *Malapterurus*.

FIG. 109. — Portion of an electroplax from *Gymnotus*, the "electric eel." *el. s.*, electric surface; *nu. s.*, nutritive surface; *el. c. t.*, electric connective tissue. Papillæ on both surfaces. (After BALLOWITZ.)

The electric tissue of *Gymnotus*, which is an eel-shaped fish inhabiting some fresh waters of South America, consists of a number of rows of electroplaxes placed vertically and face forward, to form several masses of tissue on the sides of the rear part of the body.

This tissue occupies the place ordinarily filled by muscle, and this, together with the fact that the electroplaxes are placed in a myotome arrangement, forms fairly good evidence that the tissue is derived from muscle rudiments. When we add to this that we know the electric tissues to be modified muscle in the elasmobranchs where it occurs, we have a strong confirmation of its relationship.

Each electroplax is placed with its electric surface, on which the nerve supply ends, facing directly to the rear, and the other side or nutritive surface facing forward. Both in front of and behind the plate is a layer of the electric connective tissue or jelly, the one in front being somewhat the thinner.

The posterior surface is evaginated into a large number of short, medium thick papillæ, while the anterior face is drawn out into very many very thick projections, so thick and so closely set that their sides touch, for the most part. Each papilla system may roughly be said to be as thick as the solid middle layer.

Figure 109 shows a small part of a section taken transversely to the electroplax. The cytoplasm is seen to be a delicate reticular substance slightly denser, perhaps, toward the anterior surface and especially around the edges of the spaces which separate the anterior papillæ.

The edges of the section show that over the entire surface of the electroplax there is a continuous series of short rod-like structures pointing at right angles from the electrolemma into the cytoplasm. Those on the posterior surface are the heaviest, and may represent the "stabchen" or little rods first described by Ballowitz in the torpedo.

The nuclei are not numerous when one considers the large mass of cytoplasm that they must care for. They are found near the edge and usually out in the papillæ, more in the posterior than the anterior.

FIG. 110. — Ends of two papillæ from the electric surface of the electroplax of *Gymnotus*. Nerve fibers ending in delicate mesh-shaped nerve-endings on the papillæ. (After BALLOWITZ.)

The nerve fibers approach the electroplax in the posterior jelly layer as medullated fibers, which divide and send non-medullated branches to the ends of the posterior or electric papillæ. Here the nerve forms its motor endings, consisting of a thick, heavy plexus that embraces the upper part of the papilla lying in an intimate contact with its cytoplasm. Figure 110 shows a surface view of the ends of two of the papillæ with their relations to the nerve supply.

Comparing this organ with the electroplax of *Raja*, which we hold to be the most rudimentary and complete electroplax, it can be seen that it is highly differentiated. The striation is absent, which shows specialization, and the points of contact with the ends of the nerve fibers are multiple, which seems to argue for a multicellular origin for the electroplax.

The electric tissue of *Mormyrus*, as described by Schlichter, shows some striking similarities to that of *Gymnotus*, as well as some wide differences. As in the electric eel (it is not an eel, but another kind of elongate fish) the tissue is a modified portion of the posterior, lateral, body musculature and is also composed of upright plates or electroplaxes

facing directly forward and backward. The anterior face is smooth and is the nutritive surface. The posterior or electric surface is regular and sends out a number (few compared to those of *Gymnotus*) of evaginated processes that are long and usually curved. The electric nerve applies its curious, heavy mass of endings to these processes, and in the ordinary preparations one has difficulty to say when the nerve ends and the process begins.

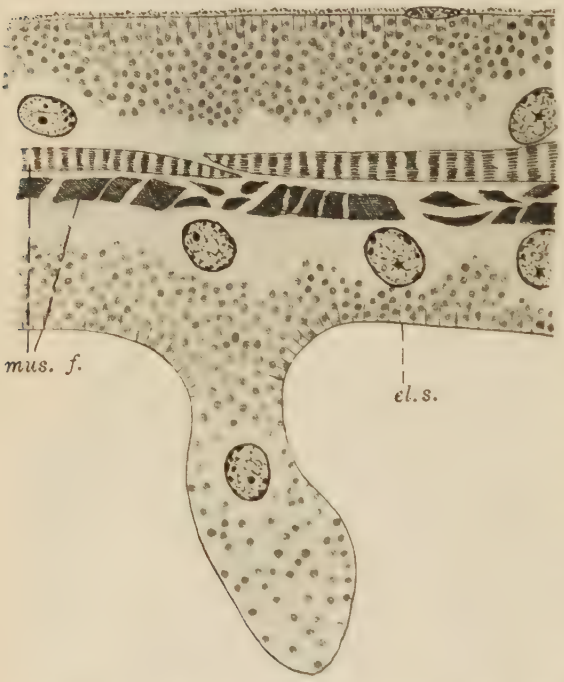

FIG. 111. Portion of an electroplax from *Mormyrus*. *el.s.*, electric surface from which part of a papilla projects; *mus. f.*, muscle fibers in middle layer of electroplax. Electric rods shown on both surfaces. (After SCHNEIDER.)

The nuclei are fairly numerous throughout the cytoplasm and are large and clear. The electric rods, as in *Gymnotus*, are found in all surfaces, even those of the processes, and they are longer and sharper than in that form. In some preparations the electrochondria are very clearly seen and are large granules resting among the rods (Fig. 111).

The most peculiar feature of this electroplax is the fact that it contains, as a middle layer, a series of small but perfect muscle bundles, which run in several directions in the plane of the electroplax. These are each composed of a number of real myo-fibrils fully striated, but in a somewhat different pattern from the striation of the regular body muscle.

In this form again we have the same two indications of a multicellular origin of the electroplax. Besides, and added to these, is the presence of the myo-fibril bundles, each of which would seem to represent the functionless remains of one of the constituent fibers that helped form the plate.

The last of these teleost forms of electric tissue is found in the fish, *Astroscopus*, which has developed what appears to have been part of an eye-muscle into its electric organ. This tissue is again composed

of flat plates or electroplaxes (Fig. 112). The parallel plates lie horizontally in the fish's head, and the upper or electric surface of each electroplax is flat and smooth and receives the nerve-endings, which are somewhat like those of *Raja* and *Torpedo* in form (Fig. 113).

The lower or nutritive layer is evaginated into a large number of long papillæ, which anastomose somewhat and project downward from the plate for about twice its thickness.

The nuclei are numerous and are differentiated into two groups, the

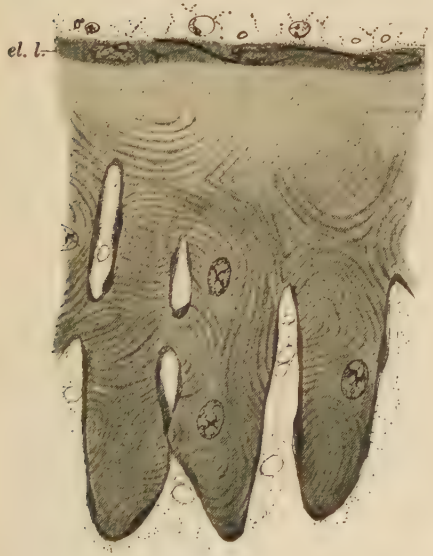

FIG. 112. — Portion of a vertical section through the electroplax of *Astroscopus*. *el.l.*, electric layer containing the electric nuclei and the peculiar fibers or rods.

FIG. 113. — Silver nitrate picture of a nerve-ending on electric surface of the electroplax in *Astroscopus*. *nv.f.*, nerve fiber. Nerve-ending is black. The reticular tissue of the electric layer shows regularly arranged spaces in which lie the transparent electric nuclei.

electric group lying in the uppermost of the three very faintly defined layers into which the plate may be divided. These nuclei are equally spaced in this layer and appear very regular in position. The second set are those few remaining ones which are scattered through the lower part of the electroplax, principally in the papillæ. The rods have not yet been certainly demonstrated in this form.

Besides the points described above, *Astroscopus* has two peculiar features. The entire cytoplasm of the electroplax is striated uniformly with a series of fine, close-set striæ that run, as in *Raja*, in curved parallel groups. This striation is probably a vestigial indication of the muscle tissue from which the electroplax was developed.

The other feature appears to be unique, and consists of a series of peculiar pointed fibers and long, pointed rods lying in the cytoplasm of the electric layer. The use of these structures, which are shaped like the classic "thunderbolts," is unknown. They might possibly be elongated "rods" such as are found in the other forms. Figure 112, from doubtful material, shows a possible thin nutritive layer which may be an artifact.

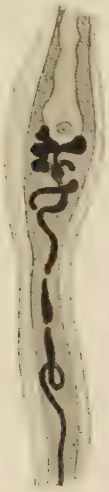

FIG. 114. — Ending of electro-motor nerve fiber on the end of the central process of an electroplax of *Malapterurus*. (After BALLOWITZ.)

Malapterurus has developed its electric cell in that part of its integument which surrounds the middle region of the body. The large, round, flat electroplaxes occupy a vertical position facing forward and backward, as in *Gymnotus* and *Mormyrus*. The surface of one of these plates is moderately regular, with the exception of the single large evaginated process which reaches backward from an anteriorly bent and cup-shaped area of the middle of the plate. This process, which is as long as a quarter of the diameter of the plate, is met by the motor nerve, which ends in its extremity, as a curled, rod-like, motor end-organ (Fig. 114). The electric surface thus faces posteriorly.

The cytoplasm of the electroplax is very lightly reticular and somewhat granular, and the large nuclei are much less numerous than in most other forms of the organ. The edge of the plate shows, in transection, a layer similar to that seen in *Mormyrus*, and almost equally distributed on both surfaces. A few granules of peculiar quality with some very few coarse fibrils can be seen in the cytoplasm near the nuclei (Fig. 115).

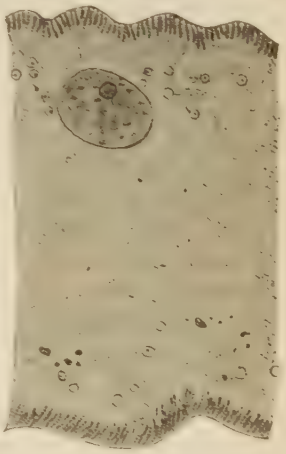

FIG. 115. — Vertical section of a portion of an electroplax of *Malapterurus*. Rods shown on both surfaces. (After BALLOWITZ.)

This electroplax has been thought to be a development of a gland cell in the skin of the fish. The writers cannot agree with this, and consider it to be, more probably, a specialized smooth muscle cell of the dermis, or even derived from a layer of the striated body musculature. The embryology and histogenesis of this electric organ should be made the subject of investigation at the earliest opportunity.

Technic. — The same as for the other electric tissues. No embryo-

logical tissues have ever been worked on in the case of the teleost fishes.

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CHAPTER X

TISSUES OF PHOTOGENESIS OR LIGHT-PRODUCTION

PROTOPLASM can produce not only heat, motion, and electricity, as was seen in the preceding pages, but it can produce light as well. This power is found in a limited number of organisms that are somewhat more numerous and much more widely distributed in the animal kingdom than are creatures that produce electricity.

The power is probably a specialization of the same or similar processes to those that produce heat, motion, and electricity. Briefly, it consists of the production of a material that, when exposed to the action of oxygen, or possibly some other substance, rapidly unites with it, and in doing so gives rise to light waves.

The light that they produce may differ in color and quantity, according to the animal that produces it, from a green light to various shades of red, purple, and violet. In some forms, several colors or shades are emitted by the same organ at different times or by different organs on the same animal. It is possible that some creatures give out a light that is not visible to the human eye, although it may stimulate the eyes of other forms whose eyes are adapted to perceive it.

The substance that thus gives light upon oxidization is of unknown chemical formula. Du Bois has extracted it from the tissue, probably in a very impure state, and has made it produce the light. He has applied the name *luciferase* to it and maintains that it is not with oxygen that it must combine to produce the light, but with another substance in the blood, to which he has applied the name *luciferine*. His theory is not considered proven, and oxygen is probably the reducing agent. This oxygen may be brought to the scene of action in some unstable compound, as hæmoglobin, for a carrier. Phosphorus forms no important part of the luciferase, as we shall call the light-giving secretion, using one of Du Bois's names without accepting his theories.

Luciferase is a secretion, the product of protoplasmic activity in changing the food materials brought to it into some specific substance of use to the organism. It can be seen in sections and teased cells, as a collection of granules that stain very readily and retain the stain with great tenacity. Sometimes it remains within the cell and is used *in situ*, the oxygen being brought to it. At other times it is discharged from the

cell to be oxidized by materials brought to it or by the free oxygen in air and water. Wherever used, it only acts properly in an alkaline medium.

The cells that secrete luciferase are of many kinds. They may be ectodermal or mesodermal in origin. In a few cases they are cells that are used mainly for other purposes, and the light-production is a secondary matter. Examples of such are the muscle cells of *Ophiura* and the blood cells of *Hystrix*. In the majority of cases, and always where the organ is of high efficiency, the cells are devoted exclusively to this function, although it can easily be seen that they originated from some of the ordinary tissues of the body, as the fat body in insects, the epithelial cells in fishes, etc.

Cells modified to make light are, in the primitive forms, epithelial in character. They appear as low or tall columnar cells. In the more highly specialized forms, and especially in the forms of mesodermal origin, they are grouped into a mass of polygonal cells. Where the cells are columnar in shape the nucleus is found near the proximal end, as in other cases of columnar gland cells, and the materials are absorbed through the proximal surface and passed in the process of elaboration toward the distal end, where they are either used to produce light *in situ* or discharged to be used outside the cell. On the other hand, where the cells are of the second or polygonal shape, the food materials are absorbed from all surfaces and the luciferase is used *in situ*, the products of combustion being passed out through the same surfaces into the blood.

Sometimes these cells are found alone in an organism, but in the majority of cases and almost always in the highly developed organs, they are accompanied by one, two, or three other and accessory tissues; the *reflecting tissues*, the *lens tissues*, and the *pigment mantles*.

The reflecting tissues are of two forms: the connective-tissue reflectors and the urate reflectors. The first of these is a very ordinary looking fibrous or thin-layered connective tissue that cannot be told in any way from a common lax or fibrous connective tissue except that it will reflect the light most perfectly. It is developed from the same tissue that forms the corium of the skin, and its nuclei are like those of other tissues of the same kind. Its fibrils or plates are usually developed at right angles to the direction of light emission. As far as can be seen, the refractive power is due to the presence of innumerable and almost invisible particles deposited in the substance of the reflecting tissue. No other tissue in the body can reflect the light in this way except the pigment found on the surface of the body in some fishes.

The second kind of reflector is made of layers of large cubical cells that have deposited in their interior, crystals of some urate that reflects the light most perfectly. This form is found in the insects and perhaps

in the crustaceans with light organs. It is fully as efficient a reflector as the connective-tissue form.

The pigment mantle is an organ whose exact function is rather obscure. It is always found on the proximal surface of the organ, and forms there a thin but perfect layer of branching pigment cells developed from a connective-tissue anlage. In the cephalopod mollusks it is formed from a few very large chromatophores of the characteristic round and flattened shape. It is apparently needless, as the reflector protects the underlying tissues from the light, and, furthermore, this pigment cannot reflect light.

The lens is often absent, even in very highly developed organs, and more particularly in those that are found in land forms. In its most primitive form it appears as a slight thickening of the usually transparent layers that are placed between the light cells and the exterior. The cells are generally of a rather flattened polygonal shape and of great transparency and refractive power. They form a lens that brings the light to a focus at a very short distance from its point of origin, and not into the parallel rays that would at first be expected.

The lens is often composed of two divisions, a proximal and a distal one, that differ from one another slightly in their texture. The meaning of this difference is not known.

The structure of these remarkable organs will be found on reflection to be remarkably like that of many of the eyes that we shall study in Chapter XIII. In each case there may be accessory tissues present to handle the light by refraction, reflection, and absorption. The two important differences are the fact that in the one case the specific cells give out light and in the other they receive it, and that in the case of the light organ there is very little direct connection with the central nervous system, while the eye is from its very nature most closely connected with it.

The nature of the light has been somewhat touched upon, but it may be well to give some rather more exact details here. It has been the subject of some very exact and convincing experiments by Langley and Very as well as Young.

Examination with the spectroscope has shown that the green light, produced by the common firefly of the United States and by several other insects, consists of those rays that have the maximum of visibility and the minimum of heat rays and ultra-violet rays. Its appearance is marked by the absence of any of the bands that show a deficiency of waves in the most actinic region. This perfection shows that nearly 100 per cent of the energy is transformed into light. The meaning of this becomes clearer when we consider that in the ordinary gas flame only a little under 2 per cent of the energy is converted into light, the rest being dissipated, so far as any use is concerned, as low heat rays! The light

of other colors, that is to be seen in some forms of animals, has not been studied as yet, and we are unable to say if these lights are as efficiently produced as is that of the firefly. As has already been said, it can easily be possible that some animals produce a light that we cannot see at all, its waves lying outside of those rays that we can see, in the non-actinic part of the spectrum.

The production of light by a living being as well as the production of electricity seems to those not accustomed to the idea as a " wonderful " process. Neither one is any more wonderful than the production of motion or of heat. In all of these cases we can easily conceive of these functions if we remember that protoplasm itself does not perform them, but is restricted to the rôle of making or secreting certain substances that, when they are brought into contact with oxygen or with each other, automatically perform the act. The great thing to understand is how the protoplasm is able to continually secrete these as well as other substances under other than some " vital " law.

Many animals give off light which is not their own, but which is produced by some bacteria which infest their bodies or which are present in the food in their digestive tracts. This can be seen in many worms and insects, especially some midges which are all aglow during flight. This condition is peculiarly true of many dead crustacea where a sudden exposure to oxygen by the turning over of driftwood and jetsam will cause them to all light up.

Examples of Photogenetic Tissues

The power of producing light is found in one-celled animals, and **the protozoön, *Noctiluca miliaris,* is probably the best known example of a luminous, one-celled animal.**

This tiny creature appears on the surface of the sea in countless numbers in some localities. Seen in the daytime under the microscope, it shows a reticular cytoplasm through which a great number of granules are scattered. These granules are probably the secretion used to produce light. We shall call them the *photochondria.* Around the nucleus is an area of cytoplasm which is undifferentiated in that it does not produce any photochondria. Its surface gives off very many fibrils, which extend radially to all parts of the periphery where they are attached. They extend through the *photoplasm,* and as the animal always contracts and shines at the same time it will not be far amiss to conclude that the *photochondria* act also as *myochondria* to the contractile fibrils, or that both kinds of granules are produced by the cytoplasm or photoplasm.

When examined at night with a low magnifying power, the animal gives off a beautiful light that appears as an homogeneous illumi-

nation. When the magnification is increased, however, it can be seen that the light is given off from myriads of tiny dots (Fig. 116). These points are probably the same as the granules or photochondria. Any stimulus, as a jar or an electric current, causes both the light to shine and the contractile elements to shorten.

The Ctenophores and other Cœlenterata also show light. — In the common *Mnemeopsis*, careful investigation has shown that the light is produced in the " ribs " on the sides of the body. It was not possible to show which cells were responsible for the secretion of a luciferase, but the substance probably appeared in some contractile elements. As in *Noctiluca*, the light appeared in response to the same stimulus that causes contraction and cilliary motion.

A case of light-production in the Echinoderms may be studied in an ophiurian. The genus *Ophiura*, of these creatures, shows two species, *telactes* and *phosphores*, which are luminous. Again, we find that there

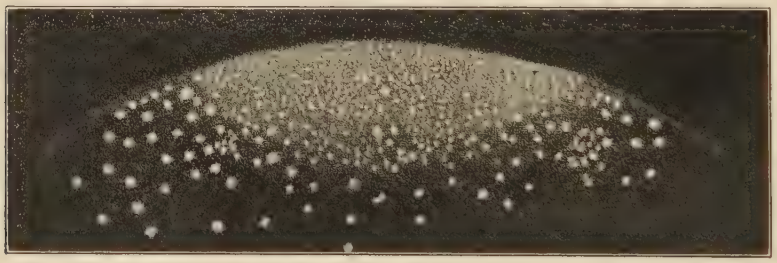

FIG. 116. — Phosphorescence in *Noctiluca miliaris* Sur (QUATREFAGES). A portion of the body is represented with numerous scintillating dots.

are no exclusively photogenetic cells. The light is produced in some muscle fibers which must secrete, therefore, both myochondria and photochondria.

The Mollusks have Many Light-producing Tissues. — All grades of specialization are shown and we shall mention three, one a superficial organ, and the others organs with an internal secretion. *Pholas*, a plecypod mollusk, is the possessor of the superficial organ which appears as a glandular epithelium that discharges a luciferase mixed in mucus. The light is given off by an external oxidization of this material and the slime continues to glow for some time after its discharge. The epithelium is found on two triangular regions on the inside of the mantle and on two cords that ascend into the siphon, as well as in some other epithelia that do not produce it strongly.

The highly specialized light-organs are found on the cephalopod mollusks, especially those which live in deep water. They show a great variety of forms, and we shall present two. One of them, **the light-organ**

of *Calliteuthes reversa*, is a well-defined and easily understood structure found on the outer integument of this dibranchiate cephalopod. Its secretion is used *in situ* in the tissues, the organ being inside the integument, instead of being thrown out as in *Pholas* (Fig. 117). Its origin is not known, and it may be either ectodermal or mesodermal.

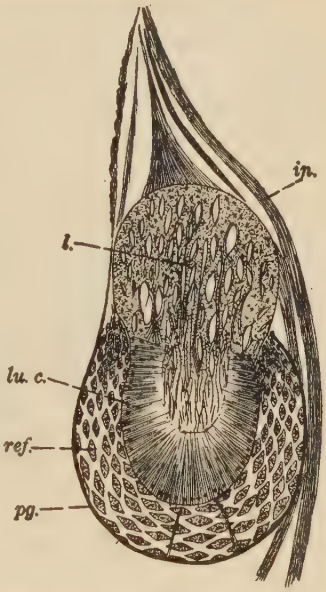

FIG. 117. — Section through main axis of light organ of *Calliteuthes reversa*. *lu.c.*, luminous cells; *l.*, lens; *ref.*, reflector; *pg.*, pigment; *in.*, integument. (After C. CHUN.)

Like most other organs of this nature, it may be described as a number of layers formed in a limited region and arranged on a distinct proximodistal axis. The first proximal layer consists of a coat of pigment, thin and black, which covers the inner end of the organ like a cap. We know nothing of its use except that it probably absorbs superfluous light.

The well defined and thick layer of tissue lying distad of the thin pigment layer is composed of the very peculiar connective tissue which has been differentiated to reflect light. The cells are spindle-shaped, and lie packed with their ends interlocked and their long axis at right angles to the direction of the light ray that comes from the nearest light cells. The substance which they have in their bodies to reflect light is possibly some urate in a very finely divided state.

Still distad of this tapetum or reflector is the photogenic layer, composed of a single row of columnar cells lying in a row that conforms to the shape of the last two layers. It is as thick as the many-layered reflector, but smaller by necessity of its distal and inner position. Its shape, as that also of the last two layers, may be roughly likened to a horseshoe. The cells which compose it are long, thin gland cells, each with a small nucleus in the proximal part, and the secretion region and storage region in the distal cytoplasm. The blood supply is evidently connected in some way with the reflecting tissue.

Distal from the light-gland is found the lens-tissue. This is rather remarkable for its apparent lack of homogeneity. It is made up of a number of heavy fibers that anastomose into a reticular mass. This mass forms two rounded and connected areas, the smaller of which lies in the concavity on the distal side of the light tissue. This small portion is directly continuous with the far larger outer or distal part, which is

made up of larger cells, and fits, as the real lens, on all the other layers combined. The reticulum is composed of meshes that are drawn out in the proximo-distal axis of the entire organ and in the direction of light transmission. If the spaces in the reticulum are filled in life with a fluid, especially if that fluid is of a high index of refraction, as it probably is, the lens would act, as a whole, very efficiently. The central axis of this organ is directed at a rather sharp angle to the body surface. It is possible that, in life, this angle can be changed at the animal's convenience.

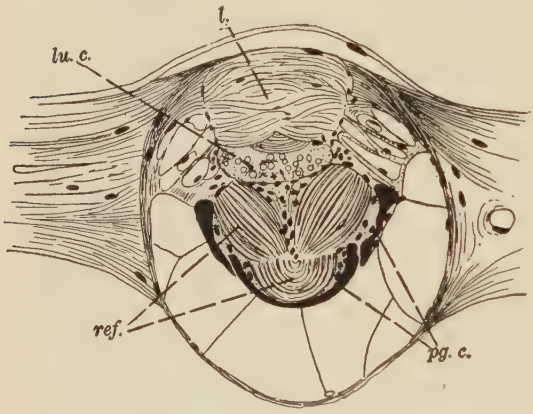

FIG. 118. — Section through principal axis of light-organ of *Abraliopsis*. *lu.c.*, luminous cells; *l.*, lens; *ref.*, reflector; *pg.c.*, pigment cells. (After C. CHUN.)

One more cephalopod light-organ must be described as an example of the highest complexity and specialization. This is **the luminous organ found in the integument of** *Abraliopsis* (Fig. 118). This organ occupies a nearly spherical sac near the surface, and has represented in its composition all the structures that are found in almost any light-organ. Its symmetrical central axis, passing from proximal to distal end, lies at right angles to the body surface. A crescent-shaped blood space occupies the posterior part of the sac. This space is divided into compartments by a number of thin connective-tissue membranes. The possibilities of blood circulation through these lacunæ are not known.

Lying between this blood space and the other central organs is a layer of pigment cells. They are symmetrically placed, one in the central axis and the other two slightly overlapping it at the side. Together they represent a cup-shaped figure, just covering and embracing, proximally, a mass of plasma which contains between its two parts the strangely formed reflectors which, like the pigment elements, are three in number.

These reflectors are a puzzle in that their positions do not seem to be mechanically adapted for the best or even for good results. They are each composed of a plate of parallel, flat cells. In the middle one, these cells are curved into a semicircle, which would be a shape of reflector not well calculated to direct the rays all outward. The two lateral bundles in our figure represent a circular reflector, which also is

seen in our section to be of a poor shape to reflect the lateral rays and those lost in the central part of the apparatus.

In the space between the pigment and reflectors is a connective tissue of unknown function. Also in the small space that lies within the arms of the reflectors we see another and somewhat fibrous connective tissue instead of the photogenetic cells as in most organs similar to this one.

The light-producing cells form a disk-like mass, somewhat thickened and very slightly concave on the distal surface. The cells themselves are not arranged in columnar order as in the last specimen, but are packed together in several thicknesses with vacuole-like spaces in the mass. The nuclei are branched and large. Cell boundaries are entirely wanting.

In the front of the light-cell mass comes the lens. It is of the same shape (a disk) as the light-cell mass, but nearly four times as thick in the proximo-distad direction. Its proximal surface is slightly convex to fit the concave surface of the light mass, and its anterior surface is the same shape to agree with the contour of the entire sac, against whose wall it lies. Its substance is made up of fibrous or plate-like cells packed in bundles, all of which lie at right angles to the axis and to the light rays. The nuclei are small and scattered.

A circular ring of reticular connective tissue surrounds the lens on all sides. It is about as thick as the lens mass, and taken together with it, forms a large disk, which occupies the front or distal part of the eye. The lens does not touch the actual body surface, but between it and the thin, tough cornea is a space, probably to contain a fluid.

Several Worms possess a Fairly Strong and Steady Luminosity.—One marine Annelid gives a bright spark during the mating season. An earthworm shows a general light given off in the slime, as is done in *Pholas*.

Light-organs are rare among the Crustacea, but are of interest because of the relationship of their bearers to the insects. Some Copepoda show a luminosity which is produced by granules of a secreted material that is thrown off. Woltereck has mentioned a possible case among the Crustacea in the deep-sea Amphipod, *Scypholanceola*, which has two pairs of peculiar organs on the head. No adequate description of the histology of these organs was accessible to the writers.

The best case of luminosity in a Crustacean is that of the Schyzopod group, *Euphusidæ*, a pelagic family with many representatives. The organ, as found in the form *Nyctiphanes Norvegica*, has been described by Vallentin and Cunningham as well as discussed by Giesbrecht, and we shall use it as a type. The light-organs, or *photospheria*, of this animal show two forms. That found on the eye pedicle is not as well developed as those found along the sides of the body. The one found on the side of the first abdominal segment is characteristic and fully developed. It is complex and highly efficient (Fig. 119).

The chief portion of this organ consists of a nearly spherical mass of tissue which is not moved by muscles, as has been asserted by some authorities, but lies loosely embedded in the tissues of the body wall and bounded distally by the cuticle. The proximal bounding layer is connective tissue, from its appearance, and is developed to act as the reflector by the plate-like arrangement of its lamellæ, which are thin, and placed in a parallel cup-shaped layer that embraces the rest of the organ on its inner end. There are but few nuclei scattered through this layer. This reflector is thin, and on its outer or proximal surface it is covered with a layer of red pigment cells.

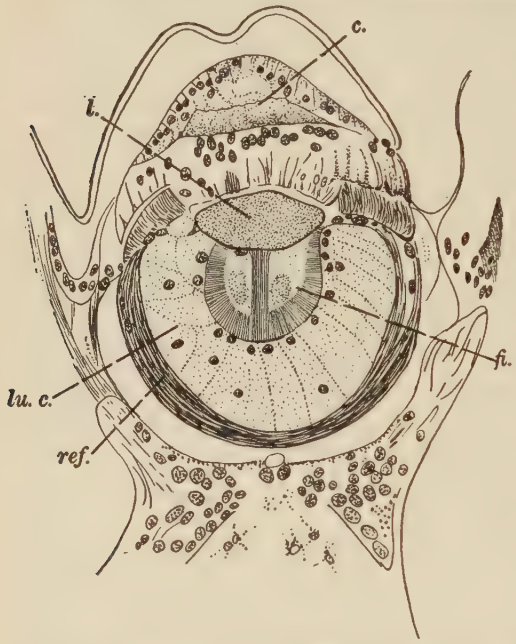

FIG. 119. — Light-organ of *Nyctiphanes Norvegica*. *lu.c.*, luminous cells; *c.*, cornea; *l.*, lens; *ref.*, reflector; *fi.*, fibrillar mass. (After VALLENTIN and CUNNINGHAM.)

The next layer distad is a columnar layer of thick, heavy cells that form a cup-like structure lying in and touching intimately, the reflector. The nuclei of these cells lie mostly distad in the layer, although some few are found proximally. This is apparently because the layer is not entirely single, but partially stratified in some places. These large cells show a cytoplasm that is full of some granular secretion, and they are probably the gland cells which secrete the light substance or luciferase. Considerable difference of opinion exists as to just which cells were the actual source of the light, and it is possible that a light substance may be produced in this layer and then discharged against the reflector or, more probably, into the inner fibrillar mass, to be there oxidized and made to shine.

This peculiar mass lies in the cup-like embrace of the last layer described, the gland cells. It is made up of several bundles crossing each other at an angle, and its outer (proximal) layer forms a series of shorter radial rods. Nuclei are very scarce in connection with it, and where found on its edge evidently belong to other tissues. Placed above this mass distally and overlapping the glandular layer is a single, very per-

fect lens. Its shape can be better understood by a glance at the figure than a page of description.

Stretched over the lens and overlapping even the reflector is a thick corneal layer which is double on its edges and single in the middle. The lower part of this double region is applied to the edge of the reflector. It is heavily striated epithelium, and continues as a sheet of loose cells, which form one of the few means by which the whole organ is connected with the tissues among which it lies. Large blood spaces effect the isolation. Above this cornea comes the hypodermis and its layer of cuticle. These structures are continuous with those on the rest of the body.

The origin of these tissues has not been worked out. Being an Arthropod, and remembering that in the insects the light tissue is developed from the mesoderm, it is plausible to consider these structures as also mesodermal. But the general appearance of the various layers, especially in a younger organ, gives a different aspect to the matter and leads one to believe that the whole organ, with the exception of the reflector, is formed by an involved invagination or delamination of the hypodermis in the embryo.

The second group of Arthropoda, the insects, exhibit several very efficient tissues that produce light. These organs are supposed to be mesodermal in origin, and probably are modified fat bodies. A reflector is usually present, and consists of a layer of closely packed cells that have the same origin as the gland cells. The light secretion is alway sused *in situ*, in the cytoplasm of the cells which produced it. It is surprising that a lens is always missing unless some of the cuticular structures may be so interpreted. While several sorts of light-organs occur, more or less simple in structure, the more complex ones may always be easily compared with the simpler. We shall begin with the study of a simple form.

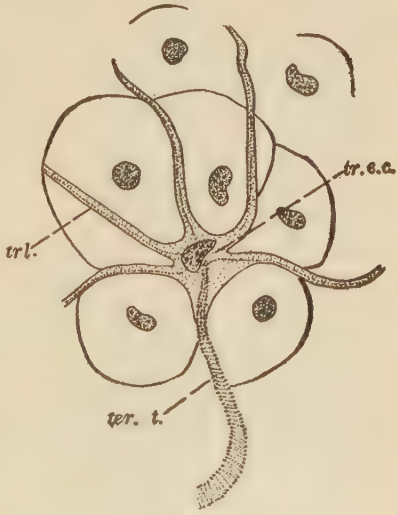

FIG. 120. — Part of a section through the light-producing tissue of *Lampyris splendidula*, showing a single tracheal end-cell (*tr.e.c.*) into which a terminal twig (*ter.t.*) of a tracheal tube enters. This twig gives off five tracheoles (*trl.*). The general histology of this tissue is much like that in the next figure. (After BONGARDT.)

The light-organ of a Lampyrid will furnish such a type, and that of *Lampyris splendidula* and *noctiluca*, as described by Bongardt, will serve our purpose.

The organ consists of a round mass of simple polyhedral cells, and is situated in the abdomen facing its ventral surface. Those of the cells which are nearest that surface are the light-cells, and secrete the light substance or luciferase in their abundant cytoplasm. The inner and dorsal layer secretes, instead, a white material (ammonium urate) and serves to reflect all light rays downward.

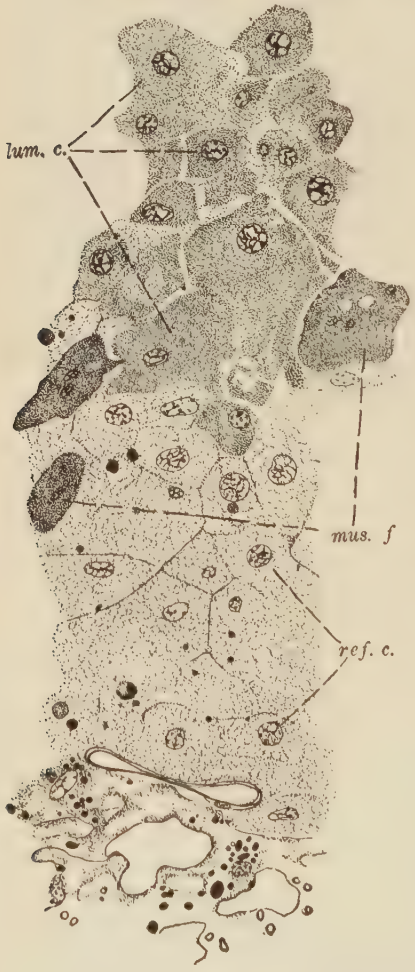

FIG. 121. — Vertical section through the median abdominal light-organ of *Pyrophorus*: *ref.c.*, reflector cells; *lum.c.*, luminous cells; *mus.f.*, muscle fibers in transection. Tracheal details not shown; they may be represented in general by those in the preceding figure. × 850.

The light tissue is most abundantly supplied with trachea to bring in air. The tracheæ branch freely, and the *terminal twigs* are distributed so that each group of several cells has one of these twigs in its midst (Fig. 120).

The relations of such a terminal twig to the group it supplies are peculiar. It enters into a central cell of the group which is called the *tracheal end-cell*, and in this cell it gives off from its end a number of fine air capillaries, the *tracheoles*, that branch out and pass through the cell group between the cells. From each end twig there are three to five or more of these tracheoles, finely ringed, and each one soon diminishes in size to a still smaller and smooth, capillary tube, which anastomoses with some of the tracheoles coming from other end-cells. In the first part of their course, at least, the tracheoles are followed and surrounded by protoplasmic processes of the tracheal end-cells. In the latter part of their way they apparently pass between the light-cells and never enter their cytoplasm. Bongardt was not able to find any tracheal nuclei in this part of their course.

The tracheal end-cells, until proved otherwise, must be looked upon as specialized hypodermal cells that have grown into the tissue with the

tracheæ to form and maintain these necessary tubes. Theoretically a thin layer of their cytoplasm should follow *all* branches of any tracheal ending. As so far actually seen, it follows these branches only part way, and the finest branches must be formed, if this is true, by the activities of the light-cells which lie in a trophic contact with them.

It should be noticed that, in this tissue, the end-cells and light-cells are irregularly grouped in alveoli of sufficient size throughout the light tissue. This is also true of the elatrid beetle, *Pyrophorus*, of Jamaica, from whose median, abdominal organ, the Figure 121 was drawn.

We shall now study briefly **the luminous tissue of our common American firefly**, *Photinus marginalis*, which has been recently worked out by Miss Townsend.

In this insect are found the same two layers, a light-producing layer next the integument, and proximal to this a reflecting layer to send out all rays. Here, too, the trachea come down through the reflecting layer and enter the photogenetic layer, but not so haphazardly as in *Lampyris*. They descend at regularly spaced intervals into little cylinders which reach vertically the whole distance from reflector to integument through the light-cell layer (Fig. 122). In their downward course they give off laterally about a hundred terminal twigs, which each pass directly into a cell lying next to the main tracheæ. These cells form the walls of the cylinder, and from the way that the lateral terminal twigs break up inside of them, we can recognize them at once as the *tracheal end-cells* that were found in *Lampyris*. The only difference is that in *Lampyris* the end-cells were scattered among the light-cells at random, so long as each one was the center of a round group of the light-cells that it could supply with oxygen, while in *Photinus* there is an organization of the end-cells into a layer that surrounds each main tracheal stem as a cylindrical tube, and outside of and between these cylinders lies the mass of light-cells.

There is here an evident structural economy. The length of tracheal tube is shorter, and the oxygen-laden air can be brought in larger quantities and more suddenly and efficiently into contact with the light-cells. The result is also evident when the insects are observed in life. The *Photinus* gives a quick, short, and dazzling flash, while a glowworm (the common ground glowworm, a lampyrid larva), with its diffuse form of tissue, glows slowly and softly for a few seconds.

Several centipedes show a light which is produced by a discharged external secretion. It is thrown and rubbed on their enemies as a slime containing tiny granules.

Among the tunicates are some members of the group that are provided with photogenetic organs. *Pyrosoma* is one in which this is very evident. The tissues consist of two cell masses in the integument, one

on each side of the body, near the siphon. The cells resemble fat cells in form, the content of the vacuole being luciferase.

The fishes are among the animals that produce light. The power is found in some simple as well as some highly developed forms in this class. As is true of the other classes, but few of the species and genera are pho-

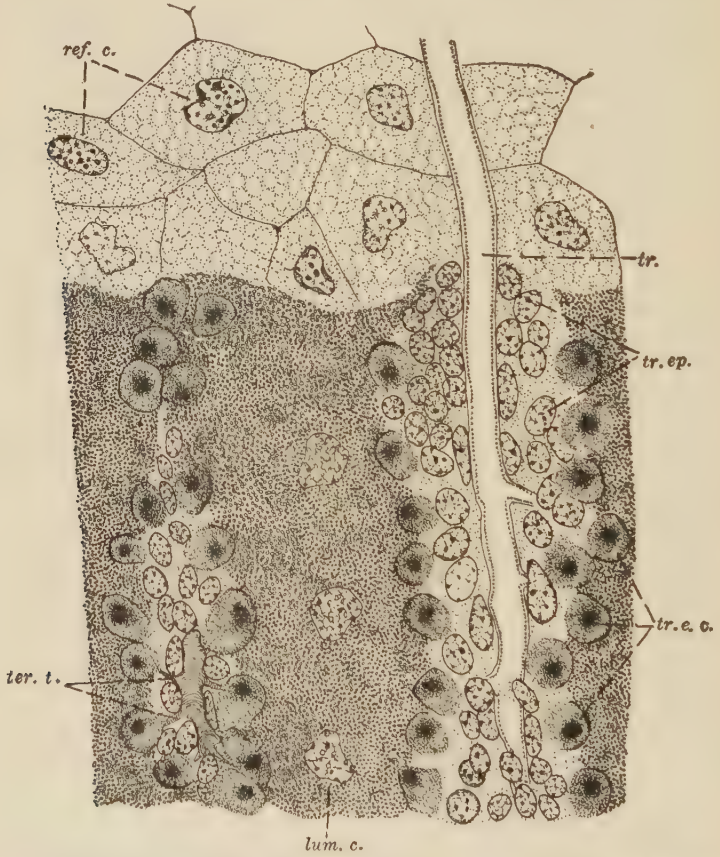

FIG. 122. — Part of a vertical section through the luminous organ of *Photinus marginalis*. *lum.c.*, luminous cells; *ref.c.*, reflector cells; *tr.*, tracheæ; *ter.t.*, terminal twig of tracheæ, tracheoles not visible; *tr.e.c.*, tracheal end-cells; *tr.ep.*, tracheal epithelium. × 1000.

togenetic, in proportion to the great numbers that exist. The organs are found in perhaps ten selachian forms and in very many more teleosts of most of the larger groups. The number of light-producing teleost species probably reaches into the hundreds.

The selachians show the simplest forms, and we shall describe, as an example, **the light tissues of** *Spinax niger*, a small Japanese shark which glows brightly in the dark. The skin of this fish shows, morphologically,

a number of regions where the light may be seen. A vertical section of one of these areas from the belly shows the epithelial structure common to most selachian fishes, a moderately thin stratified layer with large mucous cells showing in its outer part and a few widely spaced spines. The large hollow mucous cells usually contain round homogeneous concretions. The luminous organs are found lying between the spines and are much more numerous than the spines are. They can be picked out instantly as thickened regions of the epithelium into which branching pigment cells have wandered. In this region the basal layer of the epithelium is invaginated into the cutis as a pocket of simple shape with an opening that is not constricted.

The six or eight cells which occupy the bottom of this pocket are enlarged and their distal ends are collected into a central mass. Since the distal end of each one is filled by several *photochondria* or masses of light material secreted by the cells, this mass is the point from which the light emanates, and the cells are the specific photogenetic cells of the tissue. Such cells of the same basal layer as are found on the sides of the opening are not specialized, and those of their proliferated descendants which stretch across the opening of the invagination are only somewhat flattened and made transparent to permit of the light's free exit. In the specimen from which the drawing was made the poor alcohol fixation had caused

FIG. 123. — A light-organ from the skin of *Spinax niger*. *b.m.*, basement membrane of invaginated basal epithelium; *lum.c.*, luminous cells; *l.c.*, lens cells with intracellular lens secretions; *pg.c.*, pigment cells; *lum.sec.*, luminous secretion; *art.*, artifact. × 580.

some shrinking, and the artificial separation of some of the cells is reproduced in Figure 123 at *art.*

The two or three large cells, which lie above this central layer and usually also directly above one another, are specialized to form in their cytoplasm very large, solid concretions which may act as a lens to concentrate the light as it passes out. The outer of these cells are the larger, and have the concretion developed in size almost to the point that a fat globule is sometimes developed in its cell. In the inner cell the concre-

FIG. 124. — Section of light organ of a deep-sea fish, *Gigantactus*. *lum.ep.*, luminous epithelium; *ref.*, reflector; *pg.*, pigment layer; *dt.*, duct with enlarged chamber. (After A. BRAUER.)

tion is evidently in an earlier stage of development, and that brings up the question as to whether the light cells or the lens cells are renewed by growth processes or not. Their origin from a stratified epithelium would lead one to think that they were constantly worn out and replaced, while the position of the lens cells would suggest that the latter must remain where they are unless they go through a stage in which they are light cells and are finally thrown off. There is probably no renewal. A regeneration, in case of such abrasion as must often occur, is possible.

A much greater variety of light-organs is to be found in the teleost fishes. Space forbids a description of all the types, several of the most important of which will be given.

The single light-organ on the angling fin ray of *Gigantactus*, a deep-sea fish, is a simple type. It consists of an invagination of the stratified epithelium into the connective tissues of the bulb on the end of the ray. This invagination consists of a deep round sac at the fundus, an intermediate vestibule, which is wide but short, and a tube leading from the vestibule to the exterior (Fig. 124).

The deep sac is the important structure. The epithelium which lines it is still stratified, but during its proliferation each of its cells secretes the light substance, and when finally cast off at the surface it degenerates and the light substance is thrown into the lumen.

From the lumen it must be slowly forced out into the vestibule and from the vestibule it must pass into the surrounding water through the tube. It is probably oxidized in the vestibule and made to give up its light, so that only the combustion products pass into the water. The organ thus acts as an external or superficial tissue of photogenesis, a comparatively rare form, especially among the higher animals. The greater part of these teleost light-organs, however, are internal, meaning that the secretion is not discharged, but is used *in situ* in or near the cells that produced it, as was the case in *Spinax*.

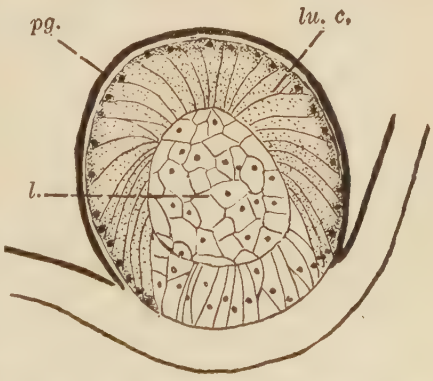

FIG. 125. — A light-organ of *Chauliodus*. *lu.c.*, luminous cells; *pg.*, pigment layer; *l.*, lens. (After A. BRAUER.)

Passing over the simplest forms that show but a few specific cells inclosed proximally by a pigment layer, we shall examine **the type of luminous organ found on** *Chauliodus*. This consists of a proximal, cup-shaped pigment mantle (Fig. 125) lined distally by a single layer of columnar cells whose small basal ends contain a nucleus and whose long distal ends are filled with the secretion.

Held in the hollow of this cup-shaped gland layer is the solid mass of cells which, in life, are transparent and refractive and act as the lens. This mass has an outer layer somewhat separated from the rest by its columnar arrangement but functionally a part of it.

Outside the lens is the connective-tissue layer of the skin. Its relation to the remainder of the organ is, functionally, that of a cornea. Morphologically the lens and gland cells came from the epithelium and

were separated from their parent tissue during the embryonic history by the connective tissue. This process has been carefully described by Greene in *Porichthys*. We shall study the luminous organ of *Porichthys*. by tracing its histogenesis, which is accessible and understood.

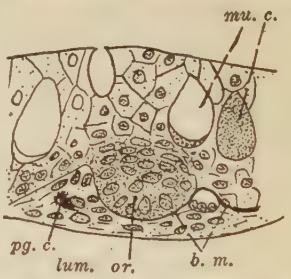

FIG. 126. — A first stage in the development of a light-organ of *Porichthys*. *b.m.*, basement membrane; *mu.c.*, mucous cells; *pg.c.*, pigment cell in connective tissue; *lum.or.*, anlage of luminous organ. (After C. W. GREENE.)

The skin of an embryonic *Porichthys* shows a thin stratified epithelium much like that of the shark. Of course no spines are present and the mucous cells are somewhat numerous.

At points in the basal layer of this epithelium a crowding of the nuclei will be noticed, and this is soon followed by an invagination of the whole layer as well as a general thickening of the epithelium at this point. Figures 126 and 127 show two invagination stages in such a region after the invagination has proceeded to some extent, and the structure resembles the light-organ of *Spinax* except that its cells are not differentiated from one another, and the outlying pigment cells, which are much increased in size and number, have not moved into the epithelium.

The process now proceeds farther than it did in *Spinax* by the constriction of the mouth of the invagination, as in Figure 128, where the rounded mass of cells is separated from its parent epithelium by the ingrowing connective tissue. Here can also be seen the beginning of a differentiation of the proximal from the distal cells of the mass. The first are becoming granular and vacuoles are appearing in their cytoplasm. Pigment cells are also present and usually more numerous than in the specimen drawn. They do not touch the invaginated mass but remain constantly separated from it by a part of the connective tissue.

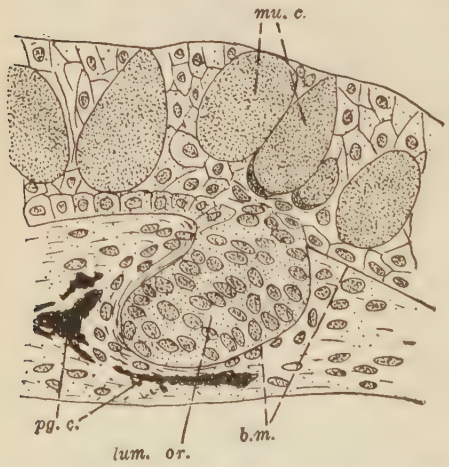

FIG. 127. — Later stage than Figure 126 of developing light-organ of *Porichthys*. Lettering the same. (After C. W. GREENE.)

This connective tissue also shows the beginning of a differentiation. It forms plates and fibers which lie parallel with the proximal outline of the invaginated cell mass and form a distinct layer.

The final development is seen in Figure 129. The invaginated cell mass is differentiated into a proximal layer which produces luciferase, and a distal portion which has grown transparent and refractive to be used as the lens. The proximal connective-tissue layer has acquired a dense white appearance that enables it to be used as a reflector, and behind it lie the pigment cells in their usual position in a light-organ.

The organ pictured in section, as Figure 129, is not yet fully formed. Greene presents a figure of an adult photosphere, but this stage in Figure 129 is sufficient for the purpose of understanding the fully formed organ, which differs from it only in size and some trifling points of form.

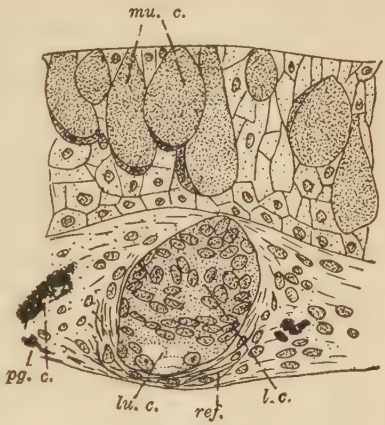

FIG. 128. — Still more advanced stage than Figure 127 of *Porichthys* light-organ. Luminous cells (*lu.c.*) of organ beginning to differentiate from lens cells (*l.c.*), also traces of reflector (*ref.*). Other lettering the same as Figure 126. (After C. W. GREENE.)

Light-organs have been described in the young of some birds, especially on the edge of the mouth in the nestlings of an Australian finch. These were rightly supposed to be of use in guiding the parent when delivering food to its young when the time or place rendered the nest dark.

FIG. 129. — Young but fully formed light-organ of *Porichthys*. *lu.c.*, luminous cells; *ref.*, reflector; *l.c.*, lens cells. Other lettering the same as Figure 126. (After C. W. GREENE.)

Chun found that a weak light appeared to be given off from the papillæ in an ordinary darkness until the room was made *absolutely* dark, when no glow whatever was apparent. Also histological investigation showed the entire absence of any cells which appeared to secrete a luciferase. It was therefore concluded that the organ was a structure that could collect

and make visible weak rays of light by reflection and, perhaps, by condensation, but which could not produce light. This same condition holds true of the eyes of many animals.

Technic. — The tissues are fairly easy to section and stain, with the exception of those that are covered with a hard chitin, which must be removed. It is best to embed, and after scraping down to the chitin, to remove it while the specimen is in the block and then re-embed. The study of some of the details has been helped by special methods. The ultimate branches of the air passages have been brought out by the use of osmic acid on the living insect. This has entered the tracheæ and blackened the tracheoles to the exclusion of all but the most immediately surrounding tissues. Also many of these tissues have been worked out from crude alcoholic material, owing to the rarity of some of the specimens. Flemming's fluid is probably the best general fixative for this class of tissue.

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CHAPTER XI

TISSUES WHICH PRODUCE HEAT

In the last three chapters we have been studying tissues that were differentiated and organized to produce three forms of energy for the use of the organism. These were *motion*, *electricity*, and *light*. Protoplasm also produces *heat*, and does it in the same way that it generates light and electricity,—by the secretion of substances that, when combined with oxygen or some other reducing agent, generate the heat required. Where known at all, the heat secretion appears in the form of granules which, could they be specifically identified, might be called *thermochondria*.

As the heat is produced by the oxidation of particles, it is probable that when first generated it is concentrated into very small areas of the cell. At this initial stage of liberation the concentrated heat must reach what would appear to us as an enormous temperature. From these points it rapidly radiates, to be distributed as lower and lower temperatures to the various parts of the tissue and body.

This heat is produced under two principal circumstances. *First*, as a step in the process of generating motion or as a by-product in the other physiological processes. This has been touched upon in discussing motion. *Second*, it is probably produced specifically and for the purpose of maintaining a body temperature.

The protoplasm of the animal body would not operate or live at the absolute zero or at any temperature between that and the freezing point. In most of the lower animals the required temperature is attained by living in a climate whose air and water furnish the heat. The organisms which live and are active in Arctic seas and on the sea bottom at great depths exist normally in a temperature that is but little above the freezing point. Others require more heat, and while they will not die for a while at or somewhat below the freezing point, they cannot live permanently unless in a temperature considerably above this. Therefore they seek a warmer climate or the rays of the sun on a rock at noonday. Countless forms, especially such as insects and reptiles, live through the cold of winter or of the tropic night, in the high altitudes, and only come out and are active during the summer time or the heat of midday.

Other animals are able to produce a considerable amount of heat by their activity. A mackerel is said to raise its body temperature eight degrees above that of the water by its vigorous swimming. In rest, however, it returns almost to the temperature of the water.

Among one group of animals only is the function found of maintaining a temperature constantly above and independent of that of their surroundings. These are the vertebrate animals, and only two divisions of these, the birds and the mammals, do this. In man the temperature is about thirty-eight degrees, while in some birds it is constantly as high as forty-four degrees. Not only are these temperatures high, but they are constant within very small limits. To secure this constancy there must be means of producing more heat when it is too low, of lowering it should it get too high, and of properly distributing it as well as retaining it in the body against radiation.

Heat-production must be stimulated by its need, by nerves which, when the temperature gets too low, automatically cause a greater production and oxidization of thermochondria. Also muscular exercise liberates much heat due to the myochondria in their work of heating the myo-fibrils to make them absorb water and contract.

This heat is distributed from its points of generation by radiation and by the circulation of the blood. When the body becomes too warm, heat is removed by the evaporation of fluids on the body surfaces. Sweat, on the outer body surface in the horse and man, and other fluids in the throats of animals like the dog and the common domestic fowl, which "pant" when too warm, are the fluids used. The extremities of these animals, as well as the surfaces, are sometimes much below the body mass in temperature.

There is but little histology to exhibit concerning this function, although there are tissues more or less set aside to perform it. As mentioned before, the muscles probably produce most of the heat, the blood distributes it, and the surfaces of the body release it and lower the temperature. Any accident or pathological condition in these may cause irregularity in the temperature, sometimes to the point of killing the organism by a reduced or excessive amount of heat.

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CHAPTER XII

TISSUES OF CIRCULATION: GENERAL CONSIDERATIONS

We have seen that the body of an organism, when it is not a single cell, is a mass of cells closely applied to each other in a manner designed to shut out most outer conditions and all foreign materials except such as may be admitted by design. We also know that all of the cells of this body, while living, must be taking in the materials used to sustain life, while at the same time they are throwing out and discarding certain other materials that are no longer wanted. The first of these materials, which we may call the food materials, must be brought into the body from the outside. They are taken in, through or between the cells that cover the body surface, or some particular part of this surface. This is very evident, as they could not enter in any other way. And it is also true that these surface cells must act as the medium through which the waste matter spoken of above is cast out. This thought serves to impress us with the importance of surface as a factor in the operations of the animal body and of the constant transfer of material that must go on in all its parts.

More important for present discussion, but not to be separated from the above ideas, is the fact that the materials, once inside the body, must be passed from cell to cell, must be distributed and must be gathered. This means that they are constantly transported. Such transportation in its simplest form is a physiological process of the cytoplasm or a physical process of osmosis or both. Lastly, it may happen that the materials pass *between* the cells instead of *through* them, and they may be assisted on their way by fluids that carry them in solution or otherwise. Such a system of passages between the cells constitutes a *circulatory system* for the distribution of materials, and the fluid used to carry the matter is called a circulatory fluid or *blood*.

It is true that if the body is so small that the materials can be easily passed from cell to cell through its mass, that there is no need for a circulatory system. And when the mass of the body becomes too great for an effective distribution from the outer surface, it is still possible to both increase the amount of surface and to make the distribution effective by a series of invaginations.

In order to understand the comparative value of such a method and of an internal transporting or circulatory system, we shall examine a

series of imaginary cellular bodies in which the surface is supposed to be everywhere equal in its power to transfer a given amount of material in a given length of time, and in which this material is likewise passed by physiological processes from cell to cell inside the body. Each cell, including the outer cells, is supposed to use what food it needs upon receiving it, and to pass the rest along equally to all of its neighbors.

Let us suppose, for discussion, that a body is the simplest kind of a mass, a cube, or better still a sphere (Fig. 130, *A*). It can then be seen that a cell situated in the center of this sphere can only receive food

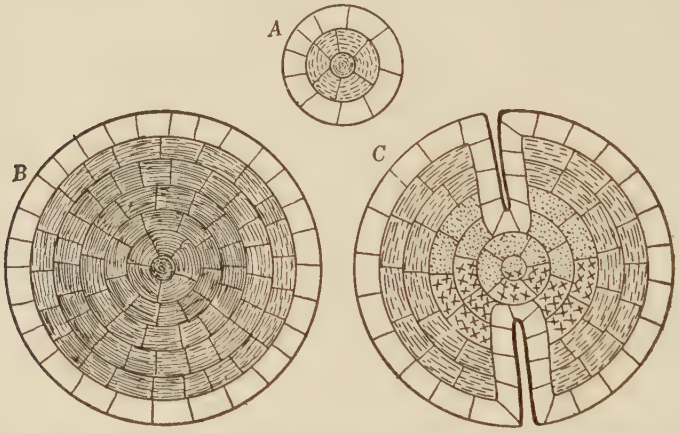

FIG. 130, *A*, *B*, and *C*. — *A*, body of cells small enough to secure necessary exchanges through surface cells. *B*, a body of cells too large to do the same. *C*, same sized mass of cells as in *B*, but with surface increased by invagination until sufficient to work necessary exchanges.

materials from the surface of the body after this food has passed through every cell that lies between it and the body surface. Suppose further that, in this spherical body, only enough material to supply three rows of cells can be passed through the outer row or layer of cells (in *A*) besides what it requires for its own use, and that in this case the organism is just able to live.

If now we suppose that the organism is larger (Fig. 130, *B*), to have, for instance, six rows of cells from center to surface, and, remembering that the surface cells are capable of supplying only three other rows of cells with food, it follows that in this case they have more than they can do, and the inner cells must perish both from lack of food and from an inability to get rid fast enough of the waste substances that are poisonous to them. The inner cells would die first, as is exemplified in the case of the cancerous growth that breaks down in its interior when it has reached a certain size and has not developed a circulation.

Two changes in the structure of the body are possible that would

serve to remedy this condition. They have already been mentioned: First, a series of one or more invaginations to increase the surface and at the same time to bring it nearer to the cells to be supplied. Second, some system of circulation of a fluid in the body to carry the materials more easily to their destinations.

Figure 130, *C*, shows a body in which the invagination of two points on its surface has supplied the need, and the materials can be sufficiently widely distributed. In Figure 131, *D*, the same principle is applied to a

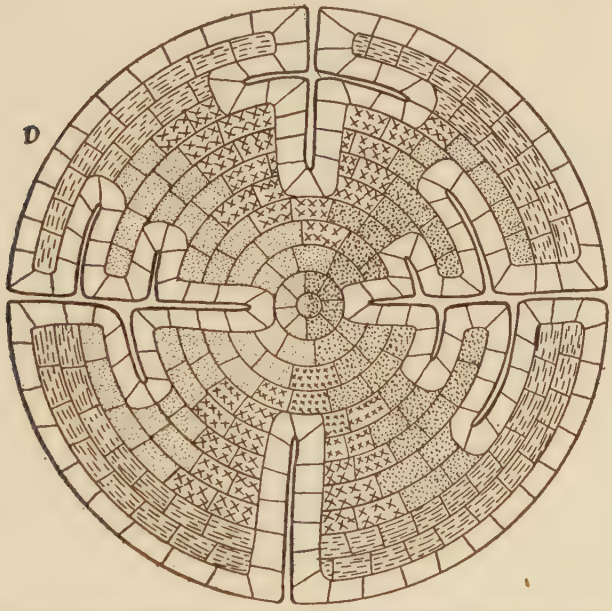

FIG. 131, *D*. — Diagram of a still larger body with extensive system of invaginations. Sufficient surface but no adaptability.

body of much greater dimensions, and it is successful as a mere distributer. But it can now be seen that in a body of any size the complexity would become very great. Perhaps of greater importance than this is the fact that each invagination would have to do exactly the same kind of work that every other one did, because there would be no way for the products of differentiated invaginations to be exchanged. An example of an animal built on exactly such lines can be found in the sponge, and such a condition constitutes in itself a form of low specialization from which there is no possible advance. Evidently invagination alone is not a change of structure by which much can be accomplished.

Turning to the second of the two changes of structure that were recognized as solutions of our primary difficulty, we must examine a body

of the same size as the one which we have just been examining, in which no invagination has been performed, but in which certain of the inner cells have been separated to form a series of communicating channels containing a fluid that can carry the materials of which we are speaking and distribute or collect them. Figure 132, *E*, shows such a case diagrammatically. This structure has solved the single problem of distribution and has relieved the cells of the burden of passing materials for such long distances through the body. But it has not created any more sur-

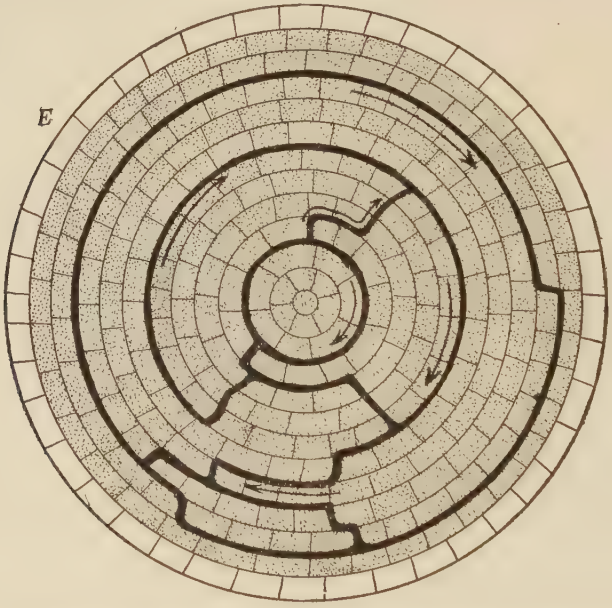

FIG. 132, *E*. — Diagram of a large mass of cells with perfect circulatory system but insufficient surface.

face for the transference of materials, and, as the original surface was not sufficient for this purpose, it can be seen that the interior cells of this organism also must die. The only difference is that the inner cells will all perish together instead of the innermost first.

Let us now consider a case where a system of invaginations to increase surface is developed in connection with a system of internal circulation to properly distribute the materials elaborated by the surfaces of the invaginations. Figure 133, *F*, shows such a condition, and it needs but a minute of thought to see that the objectionable features of either invagination alone, as in Figure 131, *D*, or of circulation alone, as in Figure 132, *E*, are solved by the combination of the two. The lack of surface in Figure 132, *E*, is provided for by the invaginations that were found

alone in Figure 131, *D*, but are combined with the circulation in Figure 133, *F*. The clumsiness and especially the lack of possible differentiation of function in the separate invaginations in Figure 131, *D*, are done away with by the circulation of Figure 132, *E*, as applied in Figure 133, *F*. One should now use the imagination and realize the fact that this arrangement can be enlarged to almost any degree of complexity and adapted to almost any need. A large number of different sorts of work can be done in the different invaginated regions, and by the extension

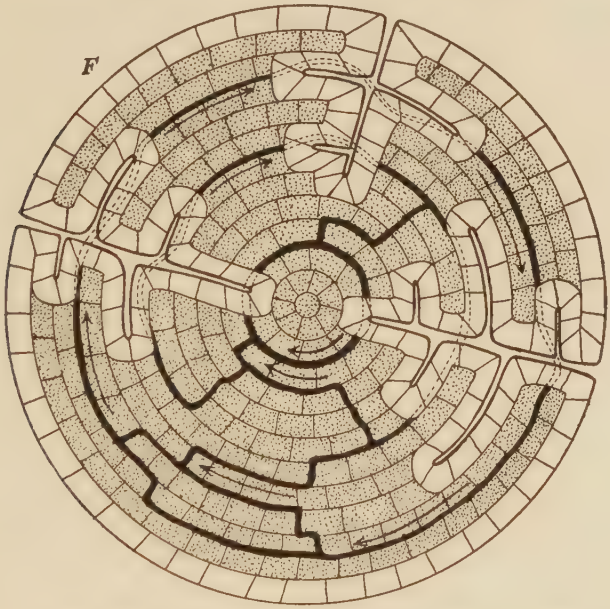

FIG. 133, *F*. — Large mass of cells with perfect circulatory system and sufficient surface.

and formation of new loops and branches of the circulatory channels the products of these activities can be carried to any part of the organism where they may be needed, or, if not needed, to any part of the body where it is possible to get rid of them.

The Figures 130 to 133 are diagrammatically correct as to relations of surface to bulk and to thickness of cell layers. In each case the source of surface supply is indicated by different formal shadings of the cells. The blood channels shown in Figures 132 and 133 are the same, and the invaginations are the same in Figure 131, *D*, and Figure 133, *F*, with the exception that the smallest invagination in Figure 131, *D*, is omitted from Figure 133, *F*, to indicate the possibility of a region remote from the surface, in a body supplied with a circulation. To preserve the same value of surface, however, the place of this smallest

invagination is taken in 133, *F*, by small additional extensions from the three remaining invaginations.

Such is the fundamental idea of the circulatory system and its relations to the system of invaginations of the original surface of the animal body. We shall now consider it apart from its relations to the differentiation of the surface functions and merely as an agent of distribution.

The circulatory channels appear in their simplest form, both taxinomically and ontogenetically, as one or more irregular spaces in the inner or mesodermal regions of the animal body. These spaces have no boundaries other than the mesodermal cells among which they lie, and some of these cells become detached and float freely in the lymph fluid that fills the lumen. Later, most of these mesodermal cells become specialized into connective-tissue cells, while many of them, in the neighborhood of the blood vessels, become modified to form the specialized walls of these spaces. Those in the fluid of the lumen may become blood corpuscles or they may also join in forming the walls. At such a period the circulatory space is not to be distinguished from other body cavities that may afterwards become separate or partly separate from it.

A low form of a specialization of this primary circulatory space is its enlargement into one or more long, continuous cavities extending the length of the body and into the limb or appendages. The blood is driven about in this set of channels by the movements of the general musculature of the body, and in some cases it exhibits a special rhythmic movement, passing first toward the anterior part of the body and then in the opposite direction. This cavity also, since it occupies the greater part of the body and contains the organs, must be looked upon as the body-cavity or cœlom.

This development, in some animals, consists of the growth of a part of the early cœlomic cavity into a long, tube-like channel with many branches and a more or less definite wall. In this tube, which may be a closed circuit or only a partial circuit, the blood acquires a continuous movement, being pumped through the system usually in one direction. At this point in its history the blood-channel system is usually separated more or less completely from a remaining portion of the cœlom, which we shall call the body-cavity. Many other differentiations and separations from these cavities occur, as the cavities in connection with the hearts and the nephridial organs, the secondary reproductive organs, and the lymphatic system and its various modifications. In the higher animals the blood-channel system arises *de novo* as a series of clefts in mesodermal tissue. There may be different regions of a circulatory system which are separate and contain different kinds of circulating media.

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TISSUES OF CIRCULATION: THE HISTOLOGY OF THE CHANNELS

The main blood-channel system itself has many differentiated regions. The region of thin-walled capillaries and lacunæ, the strong-walled conducting vessels, the blood-forming regions, and the muscular pumping stations or hearts are the chief grouping of these organs which must be treated of in more detail in the seminar part of this section. Most important, or rather most specific of these portions, are the *capillaries* and *lacunæ*, for it is here that the real work of the blood is accomplished, the exchange of materials with the tissues. This region will be spoken of as the *periphery*. Here the walls of the vessels are thinnest or even apparently wanting. In this case the connective-tissue cells that surround the channel, while not differentiated into definite channel walls, act in that capacity, so that we cannot say that retaining walls are altogether absent. The vessels of the periphery have in all cases a larger total cross section than any other total cross section in the circuit. This results in the surface of contact between blood and tissue being large enough to effect necessary exchanges of materials as well as making the current slower to give requisite time for such exchanges.

The smaller but more numerous branches of the periphery unite to form larger channels that serve to conduct the blood to other portions of the periphery, or to and from the central pumping stations, or to the blood glands. These vessels, the *veins*, together with the vessels carrying blood back to the periphery, the *arteries*, act as the long-distance carriers of the circulatory system, and their walls are usually very strongly constructed.

The pumping region comprises one or more parts of the larger channel or channels that have acquired the power of rhythmic contraction. Sometimes this region occupies a considerable extent of the larger vessels. At other times it is found in a more specialized form, occupying only a short section of the tube, but very intensely developed. Such an organ is known as a *heart*. Both of the preceding conditions may be found together, as they are in the squid and other cephalopod mollusks, where there are three or five separate hearts, and in addition the larger part of the arteries are also constantly engaged in driving the blood on its course by wave-like pulsations.

Other regions of the blood-channel system are found in which the walls are differentiated and in which the blood moves but slowly and some-

times almost comes to rest. These form the so-called *blood glands*, and in them the blood is renovated by the removal of some of its old parts or the addition of other new ones or both. There are several kinds of these organs and they will be treated of later.

Owing to the homogeneous histological structure of the circulatory organs in the various groups of animals, we shall study the detail of these organs by going through the individual system of several typical forms. It must be held in mind that the walls of these organs show a strong analogy based on the physiological (which are here mechanical) needs of the vessels. The blood fluid must be confined to the channels, and this is usually done by the single inner layer of cells, the *intima*. In some forms the intima is formed, not by the cells themselves, but by a *cuticle* which is the product of these cells (see the paragraphs on the lobster and *Imperialis* larva). The intima may alone confine the blood stream, or if the pressure is too great, it may be reënforced by the connective-tissue cells that immediately surround it. These cells develop their connective tissue as fibrils or plates or webs with which they bind and hold the vessel intact when the blood presses on its walls. Again, these primitive mesoderm cells may develop into muscle cells that surround the channel and by their contractile strength cause it to pulsate and drive the blood on its course. The arrangement of these three classes of tissues to form the wall of the vessel falls, naturally, into layers, the so-called coats of the blood vessels. Each kind of coat usually has a particular position with reference to the lumen. This position, however, is sometimes changed in the several groups for no apparent reason.

All these cells and the tissues that they form were probably not cells that were bound in the course of their development to become so specialized, but, as far as can be told, they were such of the connective-tissue cells as happened to be in the course of the developing blood channel as it pushed its way among them, and were developed in response to the needs of the vessels. Any of these connective-tissue elements would probably do the same if the blood vessel came their way, especially in the embryonic stages of the organism. This view is open to debate, however, until observation has brought proof.

We shall study the walls of the blood vessels in a few typical forms to see what variation is found among them from a histological point of view. Some Turbellarian as a primitive form; the worm *Cerebratulus*, the earthworm *Allolobophora*, the mollusk *Unio*, the mollusk *Octopus*, the crustacean *Homarus*, and an insect, *Imperialis; Amphioxus* with reference to a Tunicate, and the Vertebrate, man, with reference to a salamander, will cover the ground satisfactorily. Forms lower than the Nemerteans seldom possess a circulatory system.

The internal tissue of a Turbellarian worm is a loose aggregate of

several kinds of weakly differentiated cells, known as a *parenchyme*. These cells do not touch each other at all points, but are connected by strands, and in consequence there may be easily seen between them a great many spaces, known as the *intercellular spaces*, which are united into a large connecting system that extends throughout the body (Fig. 134).

This system of spaces is filled with a fluid and this fluid carries the digested food materials, the oxygen supply for internal cells, the combustion products, and in every other way acts as a simple blood. This is the undifferentiated and unorganized form of blood-vessel system, and a sort of circulation must inevitably take place as a result of the ordinary movements of the animal's body. This grade of structure is to be seen in a number of the lower and simpler animal forms and sometimes as an accessory apparatus to several grades of complete blood-channel systems.

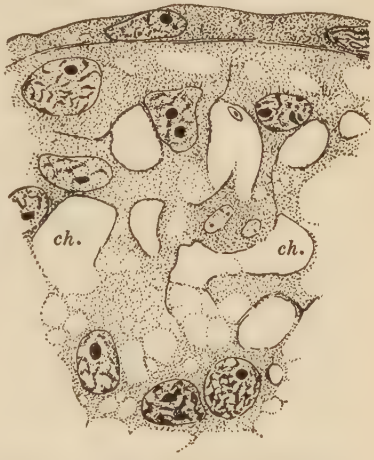

FIG. 134. — Body tissue from a small flatworm to show the intercellular clefts (*ch.*) which act as circulatory channels. ×700.

Structure of the Blood-Vessel Walls in the Nemertean Worm, *Cerebratulus*. — The location of the blood vessels is a morphological matter. They can be found for our study in the connective tissue around the digestive tract, particularly a large thick-walled vessel between the œsophagus and the rhynchocœl. A branch of this that runs circularly around the digestive tract will be studied in longitudinal sections (Fig. 135). This vessel is lined with an endothelial layer of cells that are very thin and delicate and can only be observed to advantage in well-hardened material. Otherwise one is liable to confound their nuclei with those of the blood corpuscles. When the vessel is contracted, as it is in many fixations, this layer is thrown into longitudinal folds, and the nuclei usually lie in that part of the fold that projects into the lumen. The individual cells are elongate, as are also their nuclei, and the reticulum of cytoplasm has longitudinal, drawn-out meshes that give the cell body a striated appearance in the section we are examining.

The endothelium rests upon a very weak basement membrane of so little substance that it seems to be a mere boundary in the thinner vessels, but of appreciable thickness in the larger arteries. In some fixations it seems to be radially striated, but this appearance is probably due to fine longitudinal folds and the contact of the endothelium. Directly

outside of the basement membrane comes a layer of circular muscle fibers that are remarkable for the fact that no nuclei appear in their substances or directly among them. Closer examination will show that these structures are not fibers, as are the non-striated elements of vertebrate smooth muscle, but fibrils or small groups of fibrils, each one or two of which belong to one of the large cells that lie in the layer of tissue just outside of them. Each one of these structures is most probably a group of several myo-fibrils, and not a fiber in the sense that such a structure is spoken of in the vertebrate animal, for instance. They lie three or four thick in the larger vessels and in a decreasing proportion in the smaller channels, until in the smallest there are none to be found. The cellular layer found outside of the circular muscle-fibrils consists of the cell bodies to which the muscle-fibrils belong, together with a few connective-tissue cells and an occasional nerve cell. These form an epithelial-like layer arranged radially and containing elongated nuclei. When the vessel is contracted, the cells are elongated and columnar in form, and when the blood distends the channel, they shorten and become cubical.

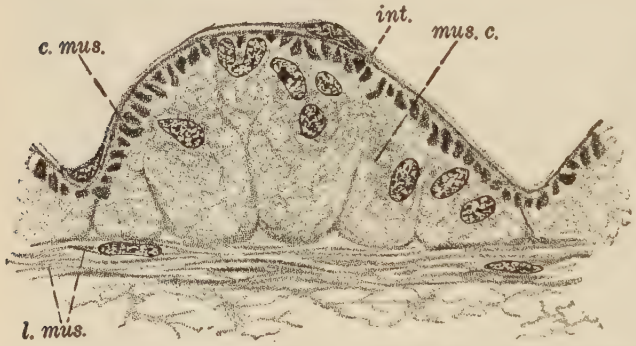

FIG. 135. — Longitudinal section of part of the wall of a blood vessel of *Cerebratulus lactatus*. *int.*, intima, a layer of cells with a very delicate membrane; *c.mus.*, bundles of circular muscle fibers; *mus.c.*, cells to which these muscle bundles belong; *l.mus.*, longitudinal muscle fibers.

The majority of the vessels possess no further covering except the very slight amount of connective tissue found around and among the muscle cells. The dorsal vessel and larger branches possess, in addition to the tissues already described, an outer layer of longitudinal muscle fibers. They appear in transverse sections of the vessel as roughly circular sections of the cylindrical cytoplasmic bodies of the muscle structures, each containing a number of the same fibril groups that we have observed in the other muscles of this animal. A single nucleus appears in each section and these are probably, therefore, syncytia. In our longitudinal sections this relation of nuclei and muscle-fibrils to the cell is not so plain, but can be grasped by a comparison with transverse sections in the same specimen.

It will be noticed of this circulatory system that it is simple and unspecialized in the fact that all its parts are substantially alike, that it is

muscular and therefore contractile for the greater part of its length, and that it lacks any of the passive connective-tissue structures found so often in systems that have a central pumping station or heart. This is because the muscle fibers perform the function that such connective-tissue cells would be required for, at the same time acting as the heart.

The Structure of the Blood-Vessel Walls in the Earthworm, *Allolobophora.* — The earthworm has a system of blood channels that are somewhat harder to understand than those of *Cerebratulus.* Owing to the delicacy of many of its structures, several diverging views are held which cannot be fully considered here.

As occurs in all blood vessels, the walls are formed by layers. The innermost of these layers is much questioned. Those who consider it as an existent formative layer of the vessel acknowledge that it is not everywhere present in the blood-channel system, but only in the larger vessels. It is described as composed of cells with flat, small nuclei, and the cell bodies form a very thin and, at parts, incomplete lining of the vessel. The cell bodies are extended in the axis of the vessel, and it is not possible to define the lines of juncture by the silver method. The opponents of this idea assert that these cells are blood cells that are clinging to the arterial walls rather than parts of the structure of the wall. We shall consider it to be an integral part of the vessel in consideration of the important part it plays in the larger vessels and " hearts " where it forms valves.

Outside of this layer, and found throughout the channel system of which it forms the real blood-retaining boundary, is a homogeneous membrane, the " basement membrane " or *intima.* This is a clearly defined and denser as well as a thicker structure than the intima found in the blood vessels of *Cerebratulus.* When the vessel is contracted, this intima is thrown into longitudinal folds. It stains red with Van Gieson's picro-acid fuchsine.

Outside again of the intima one finds two or three distinct types of structure, which distinguish the several kinds of vessels. We shall examine first the thick walls of one of the several semicircular and tubular " hearts." The inner endothelial layer is very thick here (Fig. 136). It is thrown up at several points into heavy masses which oppose each other in pairs and act as valves, one of which is shown in the figure.

The intima is very thick and is either striated or folded longitudinally or is covered on the outside by longitudinal muscle fibers. Outside of this layer come the heavy, plain, smooth muscle fibers of the circular layer. They are irregularly angular in section, with a deeply staining central mass (an artifact), and their cell bodies lie outside of them as large, well-developed cells with large, round nuclei that are only seen in a few of the cell bodies on account of the length of these latter. Outside of the muscle-

cell bodies is a layer of body-cavity cells which surround all vessels that pass through that space.

In the smaller blood vessels the place of the muscle-cell layer is taken by a layer of cells called the " wall cells," which partly encircle the vessel and are contractile in the arteries and non-contractile in the veins. They are furnished with fibrils which show, according to Schneider, a clear cross striation and must therefore be considered to be myo-fibrils.

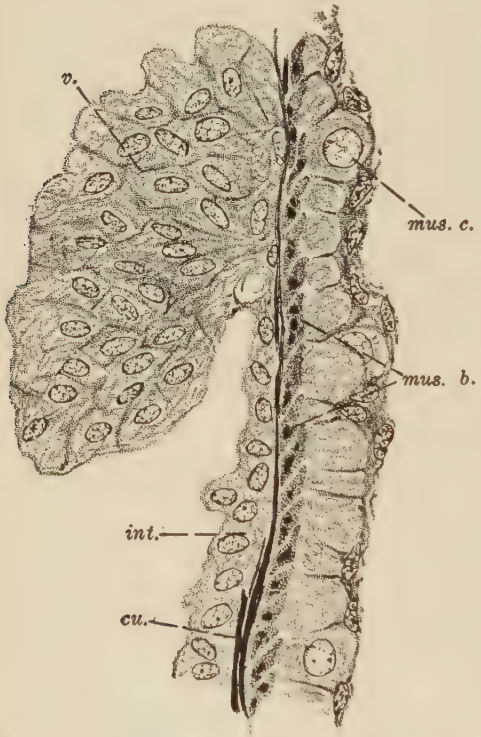

FIG. 136. — Longitudinal section of part of the wall of a " heart " in the wire-worm, *Allolobophora*. *int.*, intima, which is enlarged into a valve *v.*; *cu.*, cuticular base of intima; *mus.c.*, circular muscle cells whose fibril bundles show in transection at *mus.b.*

The Echinoderms possess a Peculiar Blood and a Very Weakly Developed System of Channels to Carry It. — The histology of their walls is extremely simple, an endothelium resting upon a loose connective tissue. These vessels widen into various lacunæ whose walls are similar.

The Mollusca all possess very definite blood channels which are differentiated into well-defined regions. **An artery (foot artery) of** *Anodonta* shows the following structure in its walls (Fig. 137). An endothelium of irregular, longitudinally extended cells forms the innermost layer. Some of these cells are supposed to become detached and form the blood and lymph cells. They are absent in some of the largest blood vessels.

This epithelium rests on a basement membrane of uniform thickness, which forms a real blood-retaining boundary. This membrane must be considered as a surface of the connective-tissue cells which are found outside of it.

Lying in the connective tissue and almost forming a boundary between it and the basement membrane are a number of single muscle

fibers which surround the vessel as a circular layer. They are smooth, and the nucleus lies on one side in the main cell body. The largest arteries show a longitudinal layer of muscle fibers which, near the heart, become irregular in arrangement as they also are in that organ.

In the Cephalopod mollusks is found a series of blood vessels that are much the same in structure as those of *Unio* or of any other mollusk. A point of importance here is the great development of muscle in all of the larger channels, veins and arteries alike. These vessels are active as pumping agents, operating without the aid of many valves. Their powerful circular muscle layer sends waves of contraction along the vessel. These waves are so strong that they close the vessel entirely and drive the blood before them.

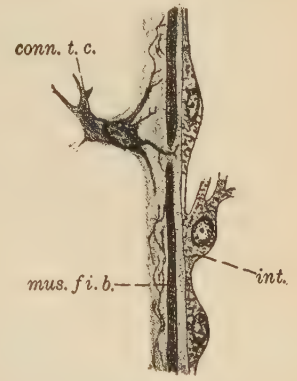

FIG. 137. — Part of a transection of a foot artery of *Anodonta*. *int.*, intima of endothelial cells on a basal membrane of weak development; *mus. fi. b.*, muscle fibril bundles (fibers); *conn. t. c.*, connective-tissue cell. (After SCHNEIDER.)

Figure 138 shows a large mantle artery from a common Florida octopus. The inner layer consists of a very thin endothelium lying on a thick, well-developed membrane. This membrane, from its position under a layer of surface cells, is a basement membrane. But also, its comparatively great thickness reminds one of the elastic membrane to be described later in a human artery. It probably is greatly strengthened and added to in substance by the connective tissue on which it lies. It lies in longitudinal folds in all but the fullest expanded arteries, and it is possibly, but not necessarily, non-elastic.

The connective tissue lying outside of the membrane is very sparse in our subject, the octopus, and can be seen to better advantage in the contracted mantle artery of a squid. Its structure has no great significance other than its function as a loose and movable connecting medium between the layers.

The muscle cells form a thick, powerful layer of circular fibers embedded in a connective tissue which holds them together and at the same time keeps them some distance apart. The individual muscle fibers are comparatively short, bluntly spindle-shaped, and reach, in the average example, about one tenth of the distance around the circumference of the artery.

The fine, sharp strands or fibrils of connective substance which bind these fibers together are loosely arranged between them except on their blunt ends. Here, these fibrils reach radially from the end of the

muscle fiber to points of attachment in all directions, especially on the sides of adjacent fibers. They do not appear on all fiber ends in the figure because many are but apparent ends, due to oblique cutting. Considering the connecting fibrils found on the surface of smooth muscle cells in other animals, it is probable that these fibrils and much of the other fibrillar material lying between the muscle cells are products of those cells and not of separate connective-tissue units.

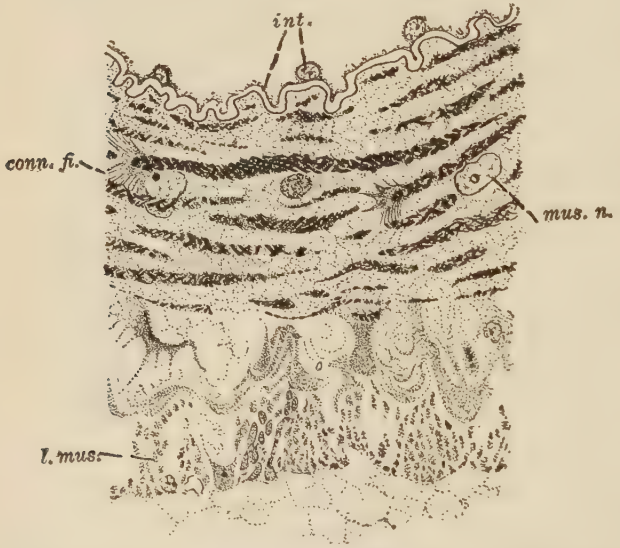

FIG. 138. — Transection of part of an artery wall of *Octopus*. *int.*, intima, consisting of a thin layer of cells lying on a thick homogeneous membrane. A muscle layer consisting of circular cells whose stout muscle-fibril bundles (fibers) show the peculiar, irregular striation found in mollusks ; *mus.n.*, muscle cell nucleus ; *conn. fi.*, connective fibrils at end of a fiber ; *l.mus.*, longitudinal muscle fibers. × 700.

The muscle nuclei are very large and lie on the sides of the fibers. In this respect they differ from the more familiar smooth muscle fibers of mammals. They are oval in form and, like other muscle nuclei, have but little chromatin and a small nucleolus.

Outside of the thick layer of circular muscle comes the layer of longitudinal muscle. It is separated from the circular layer by a region of connective tissue. Where the vessel traverses a cavity, either internal or external, the characteristic lining of that cavity is reflected over it and forms a layer external to the longitudinal muscle. The smaller vessels lose the outer layers until, in the capillaries, their only wall is a single endothelium showing evidences of its connective-tissue origin (Fig. 139).

The blood vessels of the Arthropoda are peculiar in several ways. Perhaps their most marked characteristic is a single connective-tissue epithelium that forms their chief wall. Also the fact that they are lined with an inner cuticle. We shall study one example in a crustacean and another in an insect.

The blood-vessel system of the lobster is a good one to examine, and

consists of a well-defined pulsating region or heart, large carrying vessels, capillary and lacunar peripheral parts as well as the easily studied blood glands. We shall begin with a study of the lacunar spaces (see Fig. 67). These can be found in many parts of the body, and serve to exhibit a case where the blood vessel is shown in its true light, as a cleft between masses of the connective tissue whose cells constitute its walls.

Fig. 139. — Section of a small capillary of *Octopus*, containing the slightly shrunken blood content in which two blood cells appear. × 580.

The Leidig's connective-tissue cells which border on these lacunæ exhibit hardly a trace of differentiation. The single peculiarity which they show seems to be a slight cuticular formation on the surface which they present to the blood. In the arteries which carry blood into the sinuses, a difference exists which represents the greatest differentiation of the connective-tissue walls (Fig. 140). The contiguous lining cells are smaller and have acquired a proximo-distal striation, which is thus at right angles to the vessel's surface. They also secrete a cuticle of some thickness, and this cuticle most probably is elastic during life. It is irregular, in that it contains thickened ribs which run in the direction of the vessel's course.

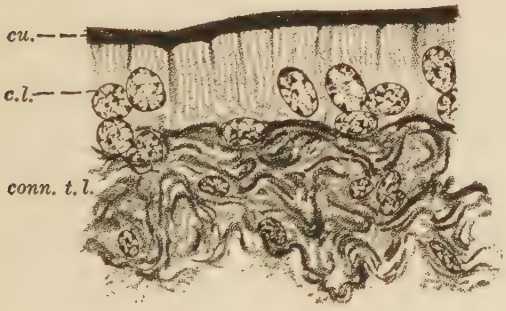

Fig. 140. — Part of a transection of the wall of a lobster's artery; *conn.t.l.*, connective-tissue layer; *c.l.*, cell lining on which appears the cuticle (*cu.*). × 700.

Those cells lying just outside of the epithelium layer are also differentiated. They have developed a great profusion of circular connective-tissue fibrils that hide the cytoplasm and among which the nuclei are, at first, difficult to see. These fibrils are either elastic or their curled and twisted arrangement permits of a spring-like elasticity, even if they are not elastic. Some of the veins show conditions which are intermediate between this and the sinus. A few of them, in particular positions, show a flat epithelium-like arrangement of the lining cells and a development of very fine fibrils directly in this first layer (see Fig. 146).

The gill capillaries of *Amphitrite* are, apparently, one of the exceptional cases where a blood vessel loses its connective-tissue covering entirely and allows the blood to flow directly among and between the cells of an ectodermal epithelium. It is still possible, however, that a delicate layer of mesodermic cytoplasm, too thin to have been heretofore detected, follows them as their true covering (see Fig. 293).

The insects have a circulatory apparatus which, considering the high specialization of the group, is remarkably simple. It consists of one very large vessel, extending for some distance in the median line of the back. It is muscular and serves to carry the blood from one end of the body to the other, and, after a very poor distribution by means of a few short vessels in its anterior region, it receives it again, as it filters its way back, through large sinuses and spaces. It then pumps it forward once more. Separate pumping organs in the limbs suffice to carry a stream into the smaller extremities.

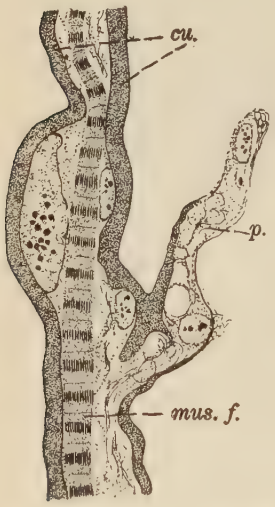

FIG. 141. - Part of a transverse section of the heart wall of an *Imperialis* larva. *cu.* cuticle, outer and inner; *p.*, process on outer side. *mus. f.*, muscle fiber. × 900.

The dorsal heart of a moth larva, *Imperialis*, is typical and consists of three layers, or five if we consider the outer and inner layers double (Fig. 141). Each of these two last layers consist of a sheet of flat cells which secrete a homogeneous and elastic cuticle on their exposed surface. The cells of the inner layer are largest in *Imperialis*, with a large central mass of cytoplasm and the remainder of the cell body so thin that it is sometimes difficult to see it. The outer layer is composed of smaller and somewhat thicker cells with smaller nuclei. This layer, on the two outer, lower quadrants of a section of the vessel, is evaginated into a series of processes which come into extremely intimate relations with a plexus of tracheal capillaries. In fact, the finest air capillaries enter in great numbers into the substance of the process. The outer cuticle is not in full thickness over these processes. The nuclei of the layer are found in the processes also.

The middle layer of the vessel consists of a single or double layer of muscle fibers. They are clearly striated and have no distinguishing cardiac features except, perhaps, a slightly shorter sarcous element. It is hard to distinguish the nuclei of these fibers from the nuclei of the outer and inner layers which bear the cuticle.

Amphioxus **has a well-defined blood-channel system** that is lined, in

all parts, with a simple endothelium resting on the surrounding connective tissues. In section, these cells appear as a line marked at intervals by the thin sections of their disk-shaped nuclei. Only on the large arterial trunk are some of the immediately surrounding connective-tissue cells developed into a single layer of muscle fibers placed in a circular position.

The tunicates show also a weakly developed blood vascular system. It resembles that of *Amphioxus* closely, and in both cases we must remember that if the animals were larger and the blood vessels consequently stronger, they would show a more definite and characteristic structure.

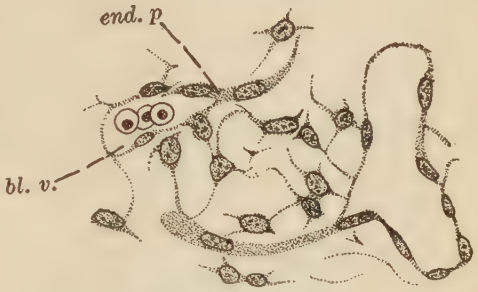

FIG. 142. — Developing blood vessels in the embryonic connective tissue of a rabbit. *bl.v.*, blood vessels containing young blood cells; *end.p.*, endothelial processes. (From "STOHR'S Text-book of Histology" by LEWIS.)

Turning to the vertebrate animals, we find the best developed and best-known forms of blood-channel structure. We shall study the walls of the vessels in man with some reference to the Amphibian forms.

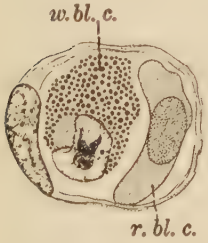

FIG. 143. — Transection of a capillary from a tadpole's tail. *w.bl.c.* and *r.bl.c.*, a white and a red blood cell surrounded by the thin wall which here consists of a single endothelial cell, showing its nucleus.

The mammal blood-vascular system begins as a system of solid connective-tissue cords which, almost immediately that they are formed, become hollow (Fig. 142). This leaves some question as to whether they were intercellular or intracellular in origin. Their subsequent development into a tube composed of many cells united into a cylindrical cover makes it appear that they were intercellular spaces from the beginning.

Figure 143 shows **a transection of a capillary in an Amphibian.** It serves well to demonstrate a case of a capillary wall whose circumference is composed of but one endothelial cell. All larger vessels show more than one such cell in section.

This covering of endothelium (Fig. 144) is all that a blood vessel actually would need to retain the blood if the pressure were always low. But owing to the weight of the blood and the great pressures that it is put to in driving it on its course, the wall of the tube is strengthened by various connective-tissue elements developed in the neighboring cells. Also the flow of blood has to be diminished at times in most localities, and this is done by

means of smooth muscle fibers which embrace the vessels. Longitudinal fibers appear outside the circular layer. These muscle fibers are the earliest additions to the vessel as it increases in size, and some of the first of them, both circular and longitudinal, are indicated in Figure 144.

This muscle tissue is added to by elastic connective tissue and white connective tissue in all larger vessels. In a well-developed artery these tissues are found in three heavy coats, each of which is subdivided (Fig. 145). The inner is known as the *intima*, and consists from within outward of the endothelial lining, a thin, flat layer of white connective-tissue elements containing a few fine elastic fibrils, and a smooth, even, and tough elastic membrane. The connective-tissue layer is drawn out longitudinally into a thick reticulum, and the elastic membrane, which is designated as the *inner elastic membrane*, is usually thrown into longitudinal folds.

FIG. 144. — Slightly oblique section of a very small artery in the marrow of a Guinea pig. To the right, the endothelial lining (*end.*) is most prominent, with the circular muscle nuclei (*cir. mus.*) cut in transection. The cell outlines of the endothelial layer were added from another preparation. A few inner longitudinal muscle cells were present. × 700.

Outside of this intima comes the thickest layer, the *media*. This is made up of circular, smooth muscle fibers and elastic fibers in a varying proportion, sometimes one and sometimes the other forming the greater part of this layer. A few longitudinal fibers sometimes occur.

The outermost layer is the *adventitia*, which begins as an *outer elastic membrane* which much resembles the inner elastic membrane. When longitudinal, smooth muscle fibers occur in an artery, they are placed just outside of this line. Outside of this we find a thick mass of white connective tissue that contains some fine elastic fibers. This layer acts more as a means of attaching the vessel to the surrounding tissues and carrying its blood and nerve supply than as a wall.

A large number of variations may be seen in the other blood vessels. Sometimes one layer is enormously developed or almost missing. Veins as a rule are deficient in muscle, though some veins, on account of their use, resemble arteries in their structure.

Most vertebrates have two separate and distinct sets of blood channels, — the one that we have been studying and **a group of somewhat smaller vessels known as the lymphatic system.** This latter has a separate and subsequent origin as a series of intercellular clefts which

appear according to McClure in close proximity to some of the early blood vessels, which latter they subsequently crowd out of existence as active blood channels. According to Miss Sabin, lymph channels are developed as outgrowths of veins in the throat and in the inguinal region.

Like the blood channels, the lymph channels are lined with a flat endothelium. Unlike the blood vessels, they do not acquire the thick muscular and elastic walls, because there is no great pumping pressure on them and the weight of the lymph is not so great. Where any approach to size is found, the strengthening of the wall is arranged as it is in a smaller vein.

The lymph channels communicate with the blood channels directly at a few points where the lymph, bearing food materials, pours into the blood. It also is connected with the blood space by temporary clefts through which plasma and lymph cells can pass, but not the red blood corpuscle.

The lymph vessels possess valves which are usually double and are evaginated folds of the walls. They also have regions where the pressure of muscles on enlarged parts of the channels causes, with the aid of the valves, a slow and irregular circulation.

FIG. 145.—Portion of a transection of an artery from man. *int.*, intima; *med.*, media; *ad.*, adventitia. (From "STOHR'S Text-book of Histology" by LEWIS.)

Technic.—The technic is usually the simplest. Flemming's fluid and paraffin sections are the best to use for the general kinds of blood vessels. Silver nitrate may often be used for the demonstration of the epithelial layers on the outer and inner surfaces.

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THE CIRCULATING MEDIA: BLOOD

Being mostly a fluid, the study of the blood from a physiological and a chemical point of a view would be more enlightening than to find out what can be known of it from its histological structure under the microscope. Physiological study of this fluid, assisted by chemical studies and certain structural studies, have taught us that some of its functions are, roughly speaking, the following: —

The primary transportation of nutriment from the digestive tract and its distribution in the tissues; the secondary transportation of food materials to and from storage in the liver and elsewhere; the transportation of oxygen from the organs of respiration to the tissues; and lastly, as a result of the use of these materials, the carrying of the products of combustion, carbon dioxide, and urea, to the respiration organs and the nephridia respectively, to be cast out as waste matter. More mechanical in its operation is the function of providing transportation, by floating, for many moving cells, the blood corpuscles, that have duties to perform in other parts of the body, and lastly, the blood has the power of coagulating into a more or less hard mass, for the purpose of preventing hemorrhages when the circulatory system is cut or injured at any point.

In its origin the blood must be looked upon as derived from the mesenchymal tissue, part of whose cells formed its walls and others the blood cells or corpuscles. By some investigators it is thought that the plasma as well as the blood cells are derived from the excavated interiors of chains of these cells, called the *vaso-formative* cells. It is possible that the blood elements are derived from certain of the mesenchyme cells that lay between the others, which became the walls of the blood channels.

The blood of different animals varies much as to which of the various functions it performs and how it performs them. We shall take up some of these functions and discuss the method of executing them in the various ways in different bloods, particularly as to the structural features employed.

The carrying of food materials (other than oxygen) is a principal function of the blood. It can only do this when the foods have been properly prepared by the digestive processes. Otherwise the food matter might seriously interfere with the performance of other duties. The degree of this preparation varies in different animals. Most foods are carried as a solution. Some are transported as an emulsion, and sometimes the blood cells carry solids, taking them into their cytoplasm and

moving along with the current. The blood platelets, which are small portions of protoplasm derived from the cytoplasm of other cells, may represent food carriers in the mammal blood. Some lower animals probably carry most of their food in the blood as a solution. The food material may bear some physiological relation to the blood, which must maintain certain conditions in order to properly function.

The blood usually carries the oxygen supply from the respiratory surfaces to the tissues which use it. The oxygen is obtained by the blood at these places by the chemical affinity of certain of its constituent substances for oxygen when they lack a certain proportion of it and are in the presence of any substance that contains more than a certain proportion of it in the free state. These substances also give it up to the tissues with which they come in contact and which need it.

One of the most prominent of these oxygen-bearing materials in the blood is *hæmoglobin*, which exists in the red corpuscles of the vertebrates and free in the blood plasma of some invertebrates. Some of the invertebrates have other substances with the same unstable affinities for oxygen that hæmoglobin has. There are several such substances. They have other colors and are probably not as efficient as hæmaglobin. That of some mollusks is called *hæmocyanine*. Sometimes the blood does not have the carrying of oxygen to do, the fresh air being conducted all over the body by fine air tubes that bring it directly into contact with the tissues. The insects show such a condition.

The carrying of carbon dioxide, as a waste material from the tissues to the respiratory surfaces for discharge, is also a duty of the blood in all forms of animals except the insects, where it is discharged directly into the air tubes. It is carried in the blood as a gas in solution.

The uric acid and its compounds are probably always carried in solution by the blood, which always has a certain percentage of them in its body. The blood is being constantly relieved of some of this burden when it passes, in parts of its course, regions where the outer surface of the vessels is lined by an epithelium that can extract this harmful matter from the blood and pass it into spaces that communicate with the exterior for its discharge.

The coagulation of the blood fluid for the purpose of closing any accidental break in the vessels is brought about by the formation of threads of fibrin in the blood of vertebrates or of colloid masses in the blood of some invertebrates, or even by the gathering together of amœboid corpuscles which intertwine their processes together to form an obstruction to the escaping blood in still other of the lower animals. These conditions occur automatically in cases where the animal is in health, and the direct stimulation which brings clotting to pass, seems to be the exposure to the air and the arresting of the blood stream, because this

must occur in a degree before the clot can form. The process is not well developed in many animals, to which a cut or other injury becomes a serious matter. In the Crustacea there is a special arrangement connected with most of the limbs that greatly aids the clotting. This consists of an invaginated ring of the integument, leaving only a small opening through which the artery and nerve pass. When the limb is injured, and the animal is threatened with a severe bleeding, it has the power to break the limb off at this point so that there is only one small artery and a vein to close, instead of a loose system of lacunæ. This apparatus also serves another purpose with which we shall not here deal.

FIG. 146. — Part of a blood channel of the lobster, containing a very coarse granular coagulum of lymph body, and blood cells (bl. c.) of several degrees of granule development. × 400.

The process of clotting often results in a change of color in the clotted blood. This color is very varied in the lower animals. It is black in most of the insects and many shades of blue or red, especially the dull shades of those colors, in the other invertebrates.

The blood corpuscle, as has been said, is a cell that is free in the blood and has assumed a variety of forms and structures according to the purposes that it has to serve in the different cases. In its simplest form, perhaps, it is a fairly large amœboid cell with a sharp outline and a good-sized nucleus that may be of very irregular shape or even multiple. The cytoplasm is abundant and filled in most cases with round granules of large size. The number and size of the granules varies in the same kind of cell and is probably dependent upon the state of activity of the cell or upon its age. Notice the different conditions of cytoplasmic granulation in the **lobster's blood corpuscles** in Figure 146; also see the same feature in the white blood corpuscles of many other animals (see Figs. 139, 143). Figure 147 shows one of the white blood corpuscles of the salamander. This shows an excessive development of these structures, and the same can be seen in many other forms. This specimen also shows another organ characteristic of the white corpuscle. This is the centrosome

and its centriole. Probably no other somatic cell that is not at or near a state of mitotic division shows this structure so well. Its sphere is free of the granules and its centriole is plainly seen, but this specimen does not show the form ascribed to it by Haidenhain, who describes the central body as a double or multiple object. The special forms of these cells found in the blood-forming glands will be described in that part.

The vertebrates have a blood corpuscle that is used solely for the carrying of oxygen, and whose cytoplasm, in consequence, is free of any granules and is saturated with hæmoglobin. In the mammals this cell is so specialized that it has lost its nucleus and the whole content is the oxygen-carrying medium lying in the plasma. These cells can be seen in the various tissues in a number of places, Figures 72 and 362 showing good examples of them.

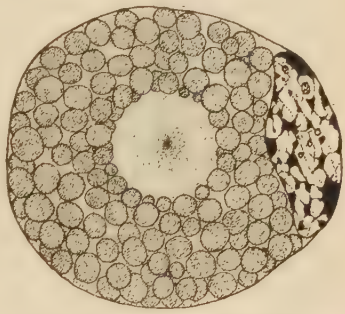

FIG. 147. — White blood corpuscle from the liver of a salamander, *Cryptobranchus*. Centrosome in middle. Nucleus to right.

In the lower vertebrates these oxygen-carrying blood cells have retained the nucleus which, however, has lost much of its usual structure and appears contracted and irregular, as is also the nucleus about to be lost by a young, red, blood cell in a mammal.

All these red blood corpuscles show a peculiar glassy and homogeneous appearance of the cytoplasm due to the contained hæmoglobin. This results in a light greenish tinge during life, which appears, however, to be red when seen in some quantity in ordinary light. Such cells stain differently from other cells unless the hæmoglobin has been entirely removed, as happens in some preparations (Perenyi's fluid). Iron hæmotoxylin stains them a jet black, fading under extreme decolorization to some dense shade of gray or green. Aniline dyes stain them brilliantly.

The red blood corpuscles of the salamander, *Diemyctylus*, can be seen in stained portions of the lung wall, as they develop from a somewhat advanced stage to the fully matured corpuscles with their contained hæmoglobin. Figure 148 shows several of these stages. It also shows that even after the acquisition of the most of its hæmoglobin, the corpuscle may continue to divide by mitotic division. From left to right in the figure are seen successively older stages, all of which are found free in the capillaries. The earlier history is not well known in this salamander.

Technic. — The preparation of the blood for microscopic study requires a vast, complicated, and delicate series of processes. There are

so many that one is at a loss where to begin. Blood should be studied both by itself and *in situ* in the tissues. For the latter, the ordinary methods are sufficient, excepting that the special blood stains, to be mentioned later, should be used here too. For study outside of the tissue,

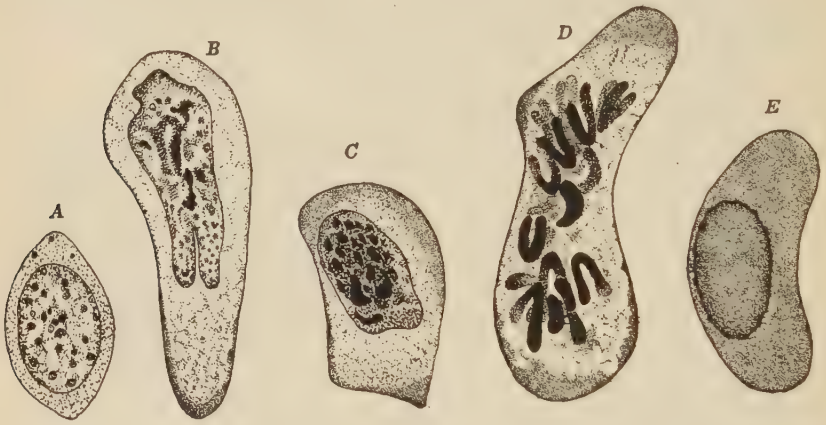

FIG. 148. — Five stages in the free life of red blood cells of a salamander, *Diemyctylus*. *A*, the youngest free stage (spindle-cell) found in the capillaries of the lungs. *B* and *C*, two successively older stages showing the slow accumulation of *hæmoglobin* in the peripheral cytoplasm. *D*, an older stage than *C*, undergoing mitotic division. *E*, fully developed and functional red blood cell. × 1000.

the blood is ordinarily taken fresh in small drops and spread, by capillary attraction, between two cover glasses, which are then slid apart and the remaining films dried and fixed at the same time by passing them through the flame of a spirit lamp. For the staining, various combinations of some of the aniline dyes are used to get a differential stain of the various sorts of blood cells that are to be found in the preparation (see Lee).

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BLOOD-FORMING TISSUES

The blood must not only take its origin from the differentiating tissues of the body and increase in amount to suit the needs of the growing embryo; but provision must also be made for producing further supplies of a tissue that is so apt to be lost in the adult by accident if not worn out by use. This idea applies to the plasma as well as the corpuscular portion, but, owing to our lack of knowledge as to the production of the

plasma, we must confine our studies to the cellular portions in the few forms in which their origin is at all understood, merely saying that the plasma is probably a secretion of some of the cells that line the channels. We shall study some of the few known blood glands and then speak of the first appearance of blood in the embryonic tissues.

In general, it may be said that the primitive mode of producing new blood cells is by a proliferation of cells from the inner endothelial walls of the blood channels, especially of the peripheral parts of the system. Such a process has often been described in many forms, usually in very general and unsatisfactory terms, however.

The first step in the specialization and organization of this function would be to restrict it to some favorable region of the channel. Such a region would be more favorable if the channel were modified in such a manner as to slow the current and provide a quiet place for the new corpuscles to be formed. This would be easiest done by an invagination of the endothelial wall of the channel, and such simple structures are to be seen in the crustacean blood glands. We shall examine one of these first.

On the arteries, especially the ophthalmic artery of the crayfish, may be found a number of small irregular glands, each composed of several short acini opening by a common and short duct into the lumen of the artery. The duct is lined by the same intima as the artery, but this structure is absent at the entrance to each acinus. Its place as a lining is taken by the blood-forming cells, which probably represent the Leidig's

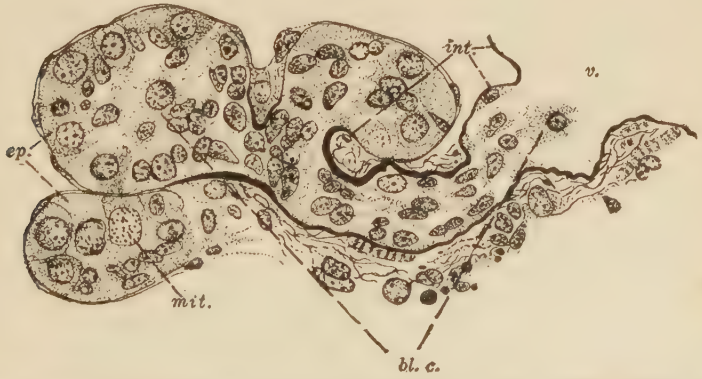

FIG. 149. — Section of a blood gland from the ophthalmic artery of a crayfish *Astacus*. *int.*, intima of blood vessel extending through the duct and partly into the acinus of the gland ; *ep.*, epithelium of gland one of whose cells is beginning a mitotic division at (*mit.*); *bl.c.*, young blood cells passing out of the gland into the vessel, *v*. (After SCHNEIDER.)

cells of the third order that line all blood-channel surfaces and secrete the intima. These blood-forming cells divide by mitosis and thus produce

many small blood cells that pass down the duct into the blood stream. They have no granules in their cytoplasm and probably develop into both kinds of corpuscles (see lobster's blood) in the different parts of the channel's system. Small striated muscle fibers are sometimes seen in the walls of the duct (Fig. 149).

A group of several other glands of somewhat doubtful meaning have been described as blood-forming glands in the mollusks. They are found in the neighborhood of the heart and are of various degrees of concentration. One is rather widely distributed through the upper mantle tissue around the heart region of *Unio* and other lamellibranch mollusks. Another is found in a more compact form at the base of the gill in *Loligo* and *Octopus*. The weight of somewhat unsatisfactory evidence has tended to show that these organs are excretory, while by some they are thought to be blood-making in function. We have treated them under the heading of excretion.

An enormous gap exists between the one, simply organized kind of blood gland found in the crayfish or the Echinoderms, and the great variety, number, and complexity of **blood glands found in the mammals.** In man these glands are used to destroy blood as well as to form several kinds of blood cells and perhaps to perform other functions as well.

The fundamental idea, that these glands are highly differentiated regions of the blood-channel wall, is difficult to maintain and, most probably, must have added to it a conception of a part of these organs as differentiated areas of mesenchymal tissues which, while in close functional relation to the blood vessels, are not morphologically a part of their walls. The exact study of the blood and the blood glands of mammals is, perhaps, the most difficult in histology as well as the one which will give the richest results, if such comparisons may be permitted. Its chief difficulty and interest lie in the fact that its cellular elements are *movable* during the same time that they are *changing*, which makes their history very hard to put together by studying dead sections. The structures cannot be studied *in situ* during life.

We shall study **a smaller lymph node, or nodule** as an example of one of the more primitive blood glands in man. Such a blood center may be found in many positions in the body and appears macroscopically as a small lump of tissue with several blood and lymph vessels entering or leaving it.

A gland of this kind begins as a differentiation of the mesenchyme in the neighborhood of some blood and lymph vessels. Branches of both kinds of vessels enter the mass. The blood vessels enter as arteries and, after forming a capillary circulation in the pulp, return as veins from the same point. This point is called the *hilum*. The lymph channels also enter into the pulp and form a wide-meshed plexus in its sub-

stance as well as a sinus-like space that extends all over the periphery. The afferent lymph vessels enter at one side of the nodule and the efferent vessels pass out at the other (Fig. 150).

A capsule of white connective tissue is formed around the whole mass, and when the nodule attains a certain size, plate-like trabeculæ of this sheath are pushed into it to provide its soft tissues with mechanical support. Elastic fibers and smooth muscle fibers may develop in

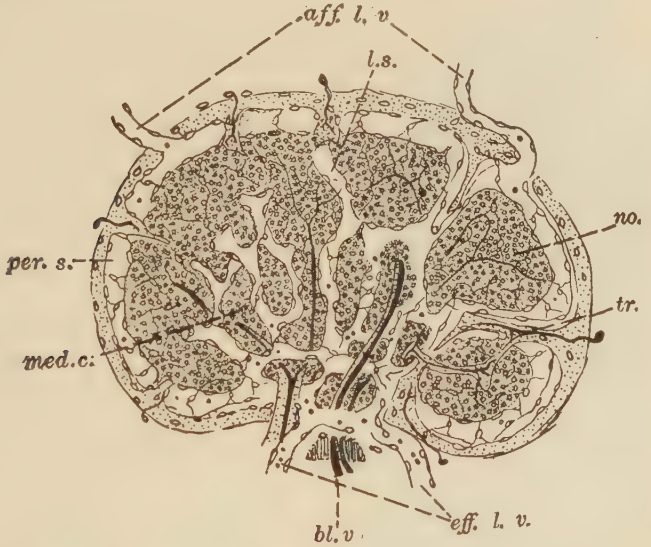

FIG. 150. — Diagram of a lymph gland. *aff.l.v.*, afferent lymph vessels ; *eff.l.v.*, efferent lymph vessels ; *bl.v.*, blood vessels ; *per.s.*, peripheral sinus ; *l.s.*, lymph sinus ; *no.*, lymph nodule ; *med.c.*, medullary cord ; *tr.*, trabecula. (From "STOHR'S Histology" by LEWIS.)

the connective tissue and trabeculæ of the larger lymph glands. The lymph sinuses usually touch the trabeculæ and follow their course. They thus come to lie between a trabecula and a mass of lymph tissue. They are separated from the lymph tissue by a layer of flat cells which may be considered to be the walls of the lymph sinus.

The excavation of the lymph-cell mass (or lymph tissue) by the lymphatics leaves it in a series of masses which when rounded are known as the *lymphatic nodules*, and when elongate as the *lymphatic cords*. The arteries and veins are found inside these masses. The lymph enters such a gland and, in flowing through its sinuses, has added to its current numerous lymph cells which creep out of the lymph mass, in which they had their origin by cell division and development, and pass out of the gland with the lymph.

Bacteria and other harmful foreign bodies may be ingested by lymph cells directly in the lymph glands. This process may go on until the usual

structural relations are so disturbed and the channels become so obstructed that the gland breaks down.

Where many lymph nodules are gathered together into a single mass they acquire a cord-like reticulum of lymphoid tissue in addition to the lymph nodules as seen in our first example. In such a lymph node the nodules are placed near the periphery and the cord mass occupies the center (Fig. 151).

FIG. 151. — A section of lymphatic tissue as it ordinarily appears. Two nodules shown. Lymph cords massed in homogeneous appearing tissue. From one of the larger masses of lymph tissue near the appendix of the cat. × 100.

The function of disposing of harmful matter extends, in the lymph glands, to the destruction of broken-down red blood corpuscles, as well as the formation of lymphocytes. This function predominates in some blood glands, which resemble lymph glands except that where lymph alone flowed into the peripheral sinuses of the real lymph gland, red blood flows into the sinuses of this kind, and they are called *hæmal glands*. Their function may be spoken of as blood filtering.

The largest gland of a lymphatic nature in the vertebrate animals is the *spleen*. This organ may be looked upon as a collection of many somewhat specialized lymph nodules lying in a much larger mass of blood-removing tissue called the *splenic pulp* or *medulla*. This splenic pulp must be compared with the peripheral sinus found in the lymphatic nodule, or more exactly and closely with the similar sinus found in the hæmal gland.

The splenic pulp is arranged in a number of radial masses, each containing several lymphatic nodules (here called *Malpighian* bodies) and each supplied by an arterial branch. A vein also collects many small branches which originate in the pulp and carries the blood out near the point (the hilum) at which the artery entered (Fig. 152). On entering the spleen pulp, each artery is closely invested with a layer of lymphatic tissue which, in man, is very thin and is expanded, at certain points only on the branches, to form the *lymph nodules*. The artery carries blood to the nodule, where some of it is diverted into capillaries in the lymphatic tissue, while the rest is carried distally by the arterial branches to the

pulp. The pulp is divided into rather indistinct regions, each of which is supplied with a fine arterial branch.

Both the nodular capillaries and the pulp branches discharge the blood into the pulp, with which it mixes and from which it is afterwards drawn out by the veins which originate in this pulp. The exact degree of direct connection between artery and vein through this pulp is a subject of much doubt and of some controversy.

The arteries show, near the point at which they terminate in the pulp, a thickened wall which is supposed to regulate the amount of blood that is discharged into the pulp. The veins show at or near their point of origin a basket-work, or open wicker-like arrangement, of the circular and longitudinal fibers, through which the blood may enter them from the pulp. These fibers are not muscle fibers, but contractile endothelial elements.

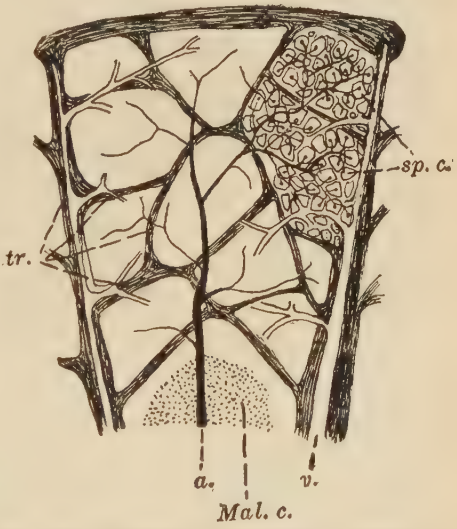

FIG 152. — Diagram of a portion of spleen. *a.*, artery with branches to the compartment units; *v.*, vein with its collecting branches; *Mal.c.*, Malpighian body or lymph node; *sp.c.*, spleen pulp cords; *tr.*, connective-tissue trabeculæ and septa. (After MALL.)

The pulp itself is composed of a reticular connective-tissue framework in whose meshes are to be found the pulp cells. The whole mass is infiltrated with the blood cells which have been thrown into it by the arteries. As may be seen in Figure 153, from a salamander, some of these red corpuscles swell up and break down and are probably ingested by white corpuscles or phagocytes.

The structure of the spleen would possibly permit of the following processes to take place in it: First, the production of lymph cells in the lymph nodules and their passage into the pulp. Second, the passage into the pulp of red blood corpuscles many of which (the broken or diseased or "worn-out" ones) are disintegrated and absorbed by the lymph cells, which then pass out through the terminal veins together with those red corpuscles which have escaped destruction.

The three (in theory but two) kinds of glands mentioned above operate to produce white blood corpuscles (*lymph tissue*) and to destroy

red corpuscles and foreign matter (*hæmal tissues* of hæmal glands and *pulp* of spleen). We shall now examine the third variety of blood gland, that which, among its other duties, is responsible for the production of the red corpuscles. This is the *red marrow* of the bones. It is developed among the fat cells of which the white or yellow marrow is composed, and consists of a few connective-tissue cells arranged as a reticulum in whose meshes the marrow cells lie. These cells are descendants of the perichondral cells that first invaded the bone during its development or during its reconstruction from a cartilage.

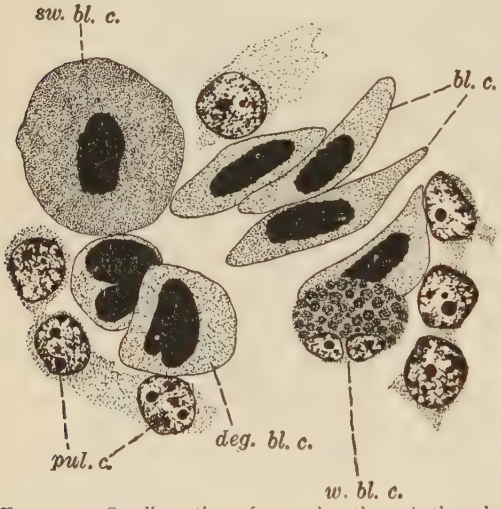

FIG. 153.—Small portion of a section through the splenic pulp of a salamander. *bl.c.*, normal blood cells; *deg.bl.c.*, degenerating blood cells; *sw.bl.c.*, much swollen blood cell about to be destroyed; *pul.c.*, pulp cells; *w.bl.c.*, white blood cell or phagocyte. × 870.

Besides the storing of nutrient materials in the form of fat, the marrow has the work of excavating the bone and sometimes of forming new bone. These two functions are treated of elsewhere, and we shall study the red marrow here with a view to understanding how it is able to furnish the blood with new red corpuscles.

The majority of cells found in a section of marrow are of medium size and possess a large round or slightly irregular nucleus with the form of chromatin reticulum ordinarily seen in young cells (Fig. 154). These are called the *myelocytes*, and they retain their numbers by mitotic division. In their earlier stages they are somewhat smaller, the nucleus is proportionally larger, and the cells are called *premyelocytes*. These cells are probably the producers of the red blood corpuscles. These are formed by the shrinking of the nucleus and its final extrusion from the myelocyte. During this time the cell is called an *erythroblast*, a *normoblast*, and finally, when the nucleus is dissolved or extruded, an *erythrocyte* or *red blood corpuscle*. The early origin of blood in the embryo is the same, except that it must come from the blood islands which are circumscribed areas of mesodermal tissue. The central cells of this tissue become erythroblasts and go through the same changes that the myelocytes do. According to Bunting "in case of extensive injury to the marrow, the

spleen may take on the hæmopoietic function, the new cells being formed in the sinuses of the organ."

Technic. — While the use of smear preparations (made as were the blood films in the last exercise) is valuable to separate and study the individual cells, it should be borne in mind that to get any real relations of the various kinds of cells to one another it is necessary to use the best sections. As this study of the structural relations is the only way in which we can understand the production of the blood, the section method should be used almost alone. The smear preparations may be used for comparison.

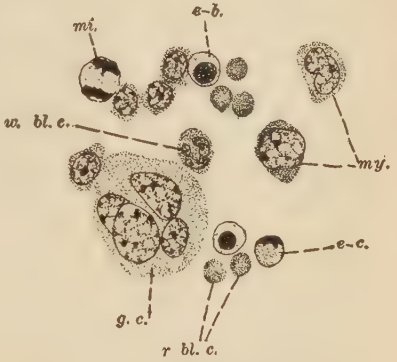

FIG. 154. — Several cells sketched *in situ* in a section of marrow from the Guinea pig's humerus. *g.c.*, giant cell ; *my.*, myelocytes ; *e.b.*, erythroblast ; *w.bl.c.*, white blood cell ; *r.bl.c.*, red blood cells ; *mi.*, mitosis ; *e-c.*, erythrocyte. × 1000.

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CHAPTER XIII

NERVE TISSUES

The nerve cells are the cells that put an organism into communication and correlation with outer chemical, physical, and mechanical conditions. In order to perform this duty they must be able to do three things: —

Firstly, to perceive or be stimulated by the outer conditions directly, or indirectly through the *stimulus* of another nerve cell, cell-product, or foreign substance, — function of *perception*.

Secondly, to transfer the stimulus, so received, as an *impulse* through the cell substance to some other surface of its cytoplasm which is in nervous contact with the cell or cells that are to be communicated with, — function of *conduction*.

Thirdly, to discharge the impulse to this other cell or cells as a *stimulus*, — function of *stimulation*.

It is probable that all cells of an unspecialized character in the lower animals have more or less of these three powers, and it is only when the cell is modified to perform the function specifically that we recognize them as nerve cells. The specialization consists of the acquisition of three different kinds of cell-organs by the cytoplasm of the respective cells: —

1. Of the development in the cytoplasm of a *perceptory organ* to receive the stimulus. This organ is a modification of the cytoplasm at some favorably situated point on the surface, and consists in different cells of a great variety of rods, hairs, plates, cones, fibrils, protoplasmic processes, etc., which are modified to suit the conditions met with (see Fig. 155, upper arrows).

2. Of the development in the cytoplasm of a number of fine fibrils, the *neuro-fibrils* and other structures to be used in carrying the resulting impulse to the other end or pole of the cell. This pole is in contact with some other cell or cells with which it is intended to communicate. The distance traversed causes the communicating cytoplasm to form a longer or shorter fiber (Fig. 155, *B*, *C*, *D*, *E*, and *F*).

3. Of the formation, at the surface of this point, of an *end-organ* or *end-plate* that is used to discharge the impulse as a stimulus to the other

cell, which may be a nerve cell, a muscle cell, or a cell of some other kind (Fig. 155, *B*, etc.). That the perceptory end-organs and the discharging end-organs are specific and necessary structures is proved by the fact that the cell cannot operate without them or when they are injured or diseased. One or both of them can be regenerated in some animals. Such a cell with its processes and end-organs is known as a *neuron*.

The nerve cell, which is usually large and well developed, may have a great variety of forms. It may be compact and but little elongated (Fig. 155, *A*), as are many surface nerve cells used to perceive mechanical

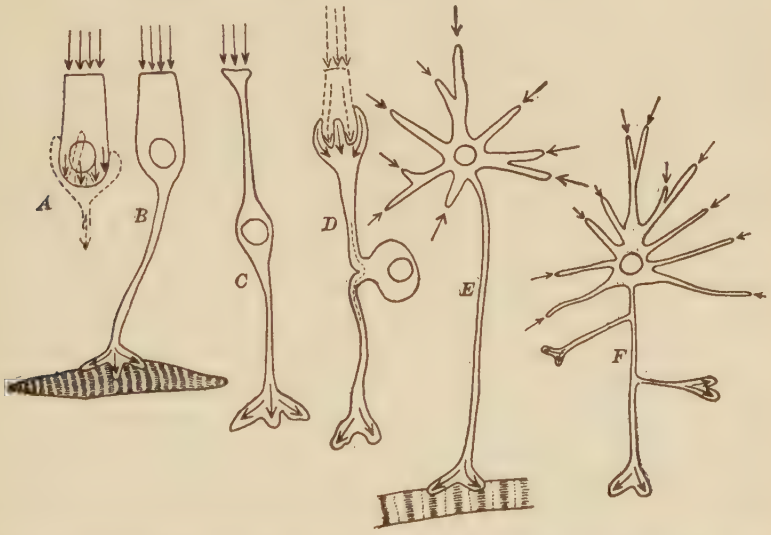

FIG. 155. — Diagrams of different kinds of nerve cells. External arrows point to receiving or *perceptory* surface; internal arrows show discharging or *stimulatory* surfaces of cells. *A*, a nerve cell with no process; *B*, the same with its discharging surface or organ on a process; *C*, both end-organs on processes; *D*, the same with impulse-path independent of the cell body; *E*, multiple perceptory organs; *F*, both end-organs multiple.

stimuli. As it is primarily intended, however, for communication between more or less widely separated parts of the body, it is almost always extended by means of drawn-out processes of the cytoplasm of its cell body (Fig. 155, *B*, *C*, *D*, *E*, *F*). The perceptory and stimulating surfaces of the cell are, of course, placed at the ends of the processes in order that they may be next to the points to be communicated with. There may be one process, placed either at the perceptory pole of the cell (Fig. 155, *A*) or at the discharging end (Fig. 155, *C*). Oftener there are two processes of unequal length, one placed at each pole. The poles and processes often are both on one side of the cell (Fig. 155, *D*). The processes are sometimes single but often multiple at one end or the other.

They frequently branch once or many times, sometimes forming a very dense and extensive network. One process or its multiple parts brings the impulse into the cell and is called the *afferent process* or *dendrite*. The impulse passes through (or by) the cell body and is carried away to its destination by the other process, which is called the *efferent process* or *neurite*. The direction of the impulse in the nerve cell is invariably the same, from perceptory end to discharging end, and it is never reversed. The nature of this impulse is not known. Its time reactions and other experiments show that it is not specifically electrical. It is not the stimulus itself carried through the cell, but a reaction of the cell to the stimulus. The impulse can be controlled and elaborated by the cell and may be retarded or suppressed or repeated in rhythmic order or even accumulated and augmented. We are thus unable to arrive at any conclusion as yet concerning the exact physical and chemical conditions that underlie the operation of impulse conduction. It is the dendrite that is usually multiple, especially in the motor cells and communicatory nerve cells that receive the stimulation from other nerve cells. It is commonly single in the perceptory or sense nerve cells.

The cell body is usually large and distinct. It is possessed of all the ordinary cell-organs necessary for its trophic maintenance and in many cases is multinuclear.

The nerve cells have been more changed in their positions in the body, perhaps, than any other tissue. These changes can also be traced better and serve to explain many features of form and function which would otherwise remain unsolved. The nerve tissues originated phylogenetically on the surface of the body and were primarily ectodermal in character. This would be a logical assumption even without further evidence, because it was only such cells as were on the outside of the body that were in contact with changing conditions which they must perceive and to which they must adapt themselves. The primitive nerve cell was probably a perceptory cell with weak powers of conduction and stimulation, which two latter powers must always follow the first.

From this superficial position all nerve cells but those that must be on the outside (or near enough to it to be accessible to the stimuli) have retreated into the most inner and best-protected positions possible. This is seen to advantage in the ontogeny of nearly all of the higher forms. The most primitive manner of retreat is for the cell body to grow down from the periphery and leave its perceptory process and end-organ at the surface. When the primitive nerve cells became differentiated to perform the three nerve functions specifically, many of the cells moved inside and took their stimuli from the perceptory cells that were still situated at the surface. The inner cells also acquired, by differentiation, new powers which have resulted in the wonderful nervous systems of many animals,

especially of man. These inner nerve cells form the central nervous system, and those remaining in direct contact with the exterior form the perceptory nervous system or sense organs.

Where large numbers of nerve cells were to be retired from the surface at once, entire parts of this surface were invaginated and the whole mass thus carried inside as in the vertebrate brain and the cephalopod brain. These form communicatory and motor centers. Many large sensory areas that can be reached inside the body (through specially developed outer tissues) by vibrations of the ether and of air or other matter are also invaginated for the protection of their delicate cells (retina, organ of Corti).

The nerve cells rarely work alone (motor cells of some jellyfish). For the most part they are arranged in chain-like pathways through the body. The links of such a chain are the individual neurons arranged with the discharging end of each one in close proximity or contact with the perceptory organ of the next, so that an impulse, beginning at the perceptory end of the first cell in the line (this must be a cell specially modified to take a stimulus from some outer conditions), will travel the entire length of the chain, ending at the discharging end of the last one as a motor stimulus to a tissue cell. The impulse may be divided and be discharged from the end-organs of a number of branches at the same time.

The circuits vary from short ones composed of two or three neurons to long ones composed of many. These paths are arranged, in some cases, so that the longer ones may be short-circuited. As some of the nerve-cells can modify and act upon the impulses that pass through them, this becomes true also of the entire circuit. Some of them are very complicated and are arranged for the performance of the mental processes in the forms that possess the power of thinking. The method of this performance is not understood.

The maintenance of these closely related pathways through the nervous system depends upon the exact and accurate working of its units, the nerve cells. These cells, called the neurons, will always, in health, carry an impulse along its appointed path and deliver it at a certain point or points. It will not allow it to "leak" into the neighboring cells or tissues until it is discharged into the cell for which it is intended, and it will always be proportional, in kind and degree, and within more or less narrow limits, to the stimulus that caused it. This proportion is not always a direct ratio. Some neurons can receive a wider range of stimuli than others which are more highly specialized and consequently more restricted in their repertory. A stimulus too weak or too strong will produce no impression whatever. The aggregate of body surfaces from which the nerve cells receive their perceptory stimuli is known in neu-

rology as the periphery; and those other surfaces, which have retired into the interior to form the ganglia and brains and receive, elaborate, and send out the reports of the sensory nerve cells as motor commands, are known as the central nervous system. This conception of the independent and exact action and interaction of the neurons is known as the "neuron theory," and is supposed to depend upon the absolute nervous separateness of each and every neuron, no matter how intimately they may be united physically. In the light of recent research it is possible that the unit of nerve activity, while usually a neuron, is sometimes a part of a neuron or even formed by two or more of them acting in unison.

Most nerve-paths have common meeting grounds with one or more others for the exchange of the nerve impulses. Here are assembled the perceptory and discharging organs of larger or smaller groups of neurons together with the cell bodies of such as have the cell body near either end-organ. Some of the neurons are confined entirely to this region, and the whole mass together with certain connective tissue and circulatory elements is known as a *ganglion*. Some ganglia are composed principally of nerve cells whose perceptory and discharging end-organs are one or both widely remote from the region, in other ganglia or at the periphery.

Some ganglia may be small and homogeneous as to the kind of cells that are found in them, others larger or containing a greater variety of cell elements. This condition is true of the greater number of animals. In some higher forms numbers of ganglia of several different kinds are collected into large central masses, which are closely assembled in some central region to form the central nervous system, as the brain in the mammals and man. The nerve cells and their products greatly outweigh everything else in such centers, other elements being neuroglia, a little connective tissue, and a considerable amount of circulatory medium. The vertebrates and cephalopod mollusks possess well-developed examples of such brains. Some of the Arthropoda are only a step behind in this respect.

A classification of the nerve tissues and their cells according to their use is the one we shall make use of here as far as possible. According to the specialization of one or the other of the three fundamental cell-organs, the cells (and the tissues) will be spoken of as *perceptory*, *communicatory*, or *motor*. Of course all nerve cells can, as has been stated, perceive, and it must be explained that, in this case, by *perceptory* cells are meant all neurons that receive *first hand* a perception of exterior conditions through a chemical or mechanical or physical stimulus. (The distinction between *physical* and *mechanical* is here used for convenience.) These three forms of cells will be considered as the *tactile* (including the *static*

and *auditory*), the *olfactory* (including the *gustatory*), and the *visual cells* and *tissues*.

Included in the communicatory cells are those that receive their stimulus from another neuron or group of neurons and transmit it as an impulse to still other neurons in a chain. Such cells are able to perceive and to stimulate other nerve cells. They exist chiefly to act as transmitting units between other neurons. As has been said, some of them can manipulate the impulse in transit, a subject we know but little about. In cases where the nerve-chain is composed of only one neuron, no such specialization has taken place, and the one cell performs all three functions. Two neurons in a chain mean that the function of communication is unspecialized. The motor neurons stimulate the muscle cells and other cells into action. This is their chief duty notwithstanding that they must also perceive and conduct. In all but the rarest cases the cell body lies in a ganglion or central ganglion as the anterior horn of the cord in the vertebrates. One exception is formed in some medusæ in which the cell body of a neuron with its perceptory organ lies in the periphery and receives stimuli which pass direct to the muscle. Otherwise the cell usually has a number of processes that receive the impulse. These are the dendrites. They may be large and branched as in the electric motor cells of fishes which furnish the stimulus to the electroplaxes found in these forms. Also in the cerebellum of man when the Purkinje cells have even more branched processes.

Technic. – Very little can be learned about the real structure of nerve tissues from the study of ordinary sections stained in hæmatoxylin and other ordinary stains. To get any idea of the real disposition of the elements one must resort to a very large number of special and difficult processes that require time and experience for their proper performance. Some of these methods will be mentioned under the several parts of the chapter, but, for the most, the reader is referred to LEE.

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THE NERVE CELL

Although the neuron is probably as highly specialized a cell as there is in the body, still it must execute the ordinary processes of assimilation, of respiration, excretion, etc., which all cells are constantly performing. It has the more of these to do, perhaps, on account of its large size and

long processes, as well as on account of its many and intense activities. It is not, therefore, all drawn out into processes, even in its most differentiated forms, but retains a large portion of its cytoplasm for the undifferentiated trophic functions. This part of the cytoplasm, the cell body, possesses in its mass and usually near the center a large, well-formed nucleus (see Fig. 156).

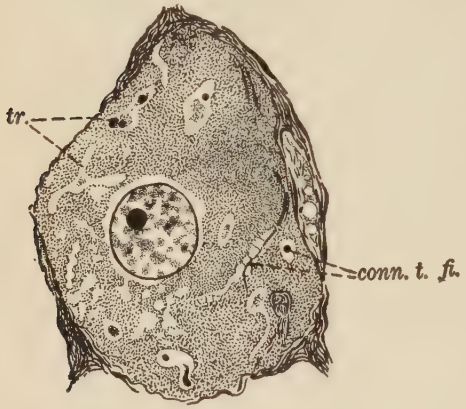

FIG. 156. — Nerve cell from the stellate ganglion of a squid, *Loligo Pealii*. *tr.*, trophospongia or lymph and blood channels; *conn.t.fi.*, connective-tissue fibrils entering to support the cell body.

The nucleus in general resembles that of an ovum or spermatogonium. It is spherical (Figs. 156, 157, and 158) or irregularly rounded (Figs. 160 and 161). The nuclear membrane is thick and sharply defined, and while a distinct linin network is not always visible, it is typical in most nerve cells, especially where the chromatin is distributed in masses of any considerable size (see Fig. 158).

The chromatin is arranged in many ways. It may be distributed throughout the nucleus in such fine particles as to appear merely as a

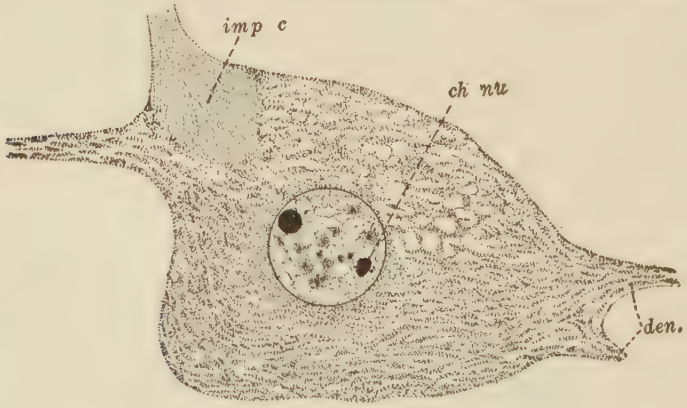

FIG. 157. — Large motor ganglion cell from the electric lobe of the brain of the torpedo, *Tetronarce*. *imp.c.*, implantation cone; *ch.nu.*, chromatin knot; *den.*, dendrites shown at their beginning. The chromatin nucleolus is always placed opposite to the nucleolus or plasmosome and the axis thus formed is the same in all the electric cells.

ground color when stained. More often it appears as a considerable number of fine but visible particles of varying sizes strung out on the linin network and especially at the intersections of the fibrils (see Fig.

158). Another common type of chromatin distribution is to be seen in the ganglion cells from the stellate ganglion of the squid and the electric lobes of the torpedo's brain where some, comparatively few, of the chromatin masses are found to be very much larger than the others and to form centers around which these smaller, dust-like particles have gathered in clouds that thin out toward the edge (see Figs. 156 and 157). In the last example, the torpedo's electric cell, still another development is to be seen in the collection of a number of these larger particles of chromatin into a dense mass of irregularly round outline called the *chromatin knot*. In this case the chromatin knot is always placed near the periphery of the nucleus and at the opposite side from the nucleolus (Fig. 157).

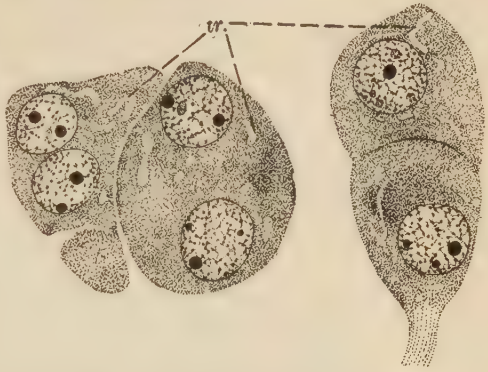

FIG. 158. — Nerve cells from a sympathetic ganglion (cervical) of the muskrat, *Fiber*. *tr.*, trophospongia, nutrient channels mostly filled with lymph. × 1000.

These various forms of the chromatin particles and the various manners in which they are arranged have not as yet given us any generalizations that enable the nerve tissue to be better understood. They show some changes during the operation of the cell and in certain diseases.

The nucleolus appears in the nerve cell much as it does in most of the other cells that have this organ well developed. It is nearly always large and sometimes very large (see Figs. 160 and 161). It is usually an exact sphere as far as the eye can determine, and only in the larger forms is any irregularity to be seen. Like other nucleoli, it sometimes contains one or more "vacuoles," or spaces containing non-staining substances. Figure 161 shows one that has a very large vacuole containing a material which shows the differentiation of a chromatic network. Non-staining nucleoli are shown in the sympathetic ganglion cells of the muskrat (see Fig. 158).

The whole nucleus is, in some instances, found at the periphery of the cell (see Fig. 164) and, while it is usually single, it may be double in some cells or even triple. This is the rule in the cells of the sympathetic ganglia of the rodents (Fig. 158), and a frequent exception in the electric nerve cells of the torpedo and the dorsal cells of the winter flounder's spinal cord (see Fig. 160).

The cytoplasm of a neuron and its processes is of particular interest

because it is through this that a nerve impulse is conducted. While it might be conceived that the impulse passed through the entire mass as through a homogeneous medium (so far as the impulse was concerned), and very much as an electric current passes through a copper wire, yet the visible presence of a fibrillar structure in parts of the cytoplasm of nerve cells drew attention to the fact that here were nerve fibrils that perhaps were specific cell-organs of conduction.

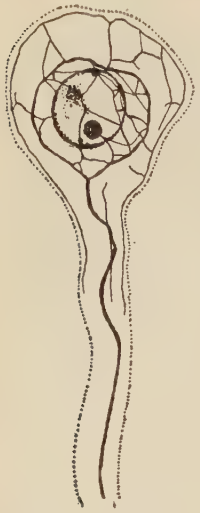

FIG. 159. — A small nerve cell from the medicinal leech, stained to show the two sets of fibrils. (From SCHNEIDER after APATHY.)

For a long time, while it was recognized that these fibrils might form such paths, it could not be proved that any one or more of them ran continuously through the cytoplasm for any distance that would warrant regarding them as such organs. Apathy and other investigators, however, learned how to stain these cells in such a manner that particular fibrils were differentiated out of the mass of fibrillar tissue and shown to run continuously over courses that were more than probably the same as those taken by the nerve-impulse. Figure 159 shows a representation of a nerve cell prepared by Apathy. His method differentiated out several kinds of fibrils. At present it is known that the several kinds of fibrils thus discovered do form possible paths of conduction. It is not fully demonstrated that these paths picked out by the stain are the only ones. It is probable that the method has picked out some fibrils physiologically or functionally different from the others just as Golgi's method selects certain neurons to the exclusion of others. Figure 159 shows a cell in which Apathy's method has demonstrated two different sets of fibrils, one of which is supposed by Apathy to bring the impulse into the cell and the other to take it out again.

Seen in most preparations, the cytoplasm of a nerve cell is ordinarily found to show, besides the fibrils, a number of easily seen masses of a granular substance and a certain proportion of undifferentiated cytoplasm, or neuroplasm, as it should be called in this case. Besides these usual features it may contain pigment bodies and other rarer structures, as a centrosphere, centrosome, cell-caps, etc. The granular substance stains very deeply in the ordinary staining reagents. Most nerve cells show it throughout the greater part of their cytoplasm as masses of a material that has taken the stain fully as deeply as the chromatin of the nucleus, while all other cytoplasm around it is very slightly stained.

These bodies are known as the *chromaphyllic masses*, the *tigroid bodies* being another and perhaps more convenient name. These masses are not homogeneous, but are composed of individual granules that appear to be characteristic organs of the nerve cell. We shall call the granules *neurochondria* to distinguish them from the other granules that are sometimes found in the nerve cell.

That the neurochondria are active organs of nerve work may be surmised from the changes that take place in them under different physiological conditions. Poisons and stimulants, as well as fatigue and disease, cause marked changes in their amount and distribution. They are possibly masses of food material for the trophic processes of the cell or

FIG. 160. — Dorsal giant nerve cell from cord of young flounder, *Pseudopleuronectes*. Three nuclei. The chromophyllic substance in two forms, a finely granular deposit in the cytoplasm and larger spindle-shaped bodies in both cytoplasm and part of the implantation cone. Fibrillar nature of implantation cone (*imp.c.*) well shown. × 700.

even to more directly support the nervous activities themselves. Not all nerve cells appear to possess these structures. It is probable, however, that, considering their almost universal presence in the various nerve cells, they exist in these apparent exceptions as diffused or invisible structures.

The neurochondria are individually so small as to be almost invisible. They are visible, usually, because they are arranged in the masses spoken of above, the tigroid bodies (see Figs. 157 and 161). These bodies are arranged through the cytoplasm (neuroplasm) with an apparent view to meeting two conditions, — a fairly general distribution and the avoiding of interference with the courses of the neuro-fibrils. This results in some such figure as is seen in Figures 157 or 161. In this last, which is a nerve cell from the cord of a fish, the distribution of the neurochondria in

masses would be clearly shown if the section should be exposed to the action of trypsin, when the other cell parts would be digested and leave only the neurochondria, which could then be studied alone and apart from any of the other cell-organs.

Certain of the tigroid masses are remarkable in their position or size. Some of them form a cap-like structure on one or more sides of the nucleus. Some extend out into the dendritic processes, always, however, becoming narrower and longer as they are found farther from the cell body. Their absence in the neurite and implantation cone must be

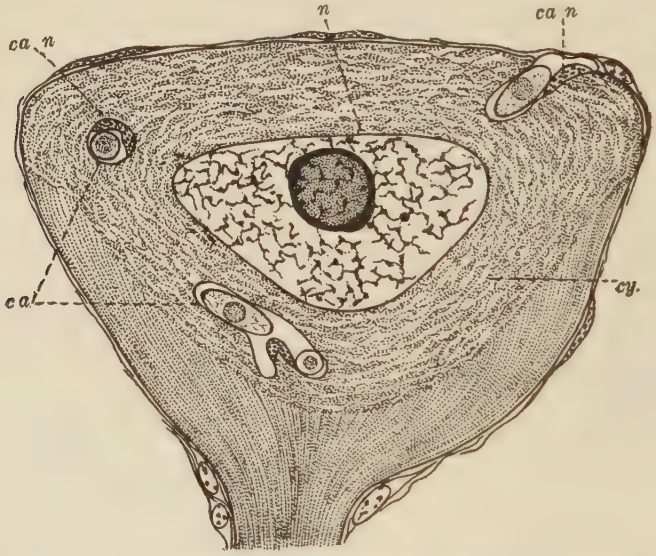

FIG. 161. — Giant dorsal nerve cell from the spinal cord of the marbled angler, *Pterophryne histrio*. *n.*, nucleus; *cy.*, cytoplasm; *ca.*, capillaries; *ca.n.*, nuclei in the capillary walls.

looked on as an economy of space, the parts in question being too small to accommodate the tigroid masses which can just as well be kept in the cell body.

In the huge dorsal nerve cells of the young of the winter flounder a peculiar set of chromatic bodies make their appearance, both on the edge of the implantation cone and extending somewhat out into the fiber (Fig. 160). These are probably a developmental feature. They are much larger than the permanent tigroid masses in the cell and are very compact and smooth in outline. They possibly are reserve stores of the chromaphyllic substance and give up their material to the growing and forming tigroid bodies. Their smooth outline and compact structure make it possible that they belong to some other group of granules in the nerve cell. Such essentially different granules are found in parts

of the cytoplasm. Some are fatty in nature and others appear to be coagulated lymph or blood that was in the cell at the time, particularly in the channels that are described below.

FIG. 162. — Large nerve cells from sub-œsophageal ganglion of *Helix*. *conn.t.*, connective-tissue elements invading cytoplasm; *gr.*, granules in the cell channels. × 300. (From a preparation by McCLURE.)

Many nerve cells are among those animal cells that approach the limit of size that a cell can attain and still have surface enough to perform its nutritive and excretory exchanges. A few go above that size and in consequence are obliged to develop in their cytoplasm a set of channels that will serve to increase this power of exchange. Among these are many nerve cells that possess lymph channels or spaces of various degrees of size and efficiency. These lymph channels can be seen, weakly developed, in the sympathetic ganglion cells of the musk-rat (see Fig. 158), and more strongly shown in the larger nerve cells of the squid (Fig. 156), in which latter form they also are occupied, in part, by the connective-tissue fibrils that penetrate the cell substance. More exceptional is the case of the giant dorsal nerve cells of the pediculate fishes, in which the capillaries themselves, with their coat of connective-tissue cells, enter the cytoplasm of the cell and supply it with a medium of exchange (Fig. 161).

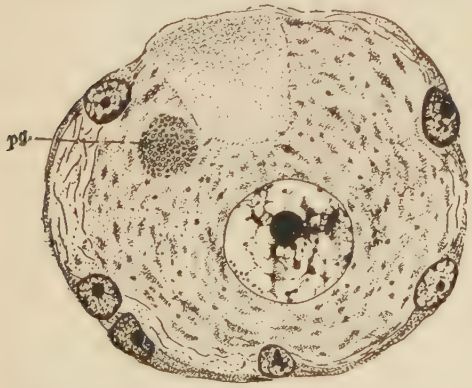

FIG. 163. — Spinal ganglion nerve cell from electrocuted man. *pg.*, pigment mass lying in cytoplasm next to implantation cone. × 1000.

It has been noted above that connective-tissue fibrils enter the neuro-

plasm of the nerve cell. The cells that produce these fibrils are some of the connective-tissue elements that have moved into the nerve tissue and, in connection with the neuroglia cells, are used to give it support and tensile strength. In some of the lower animals they form a thick cover-

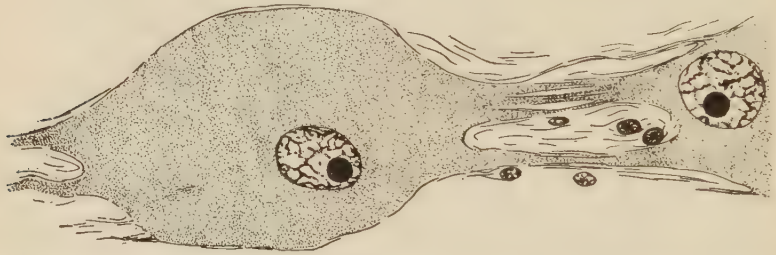

FIG. 164. — Dorsal nerve cells in cord of a flounder, *Achirus*. Shows cytoplasmic connection between cells and an eccentrically placed nucleus. × 800.

ing for the cell, and their fibrils are so woven into the outer texture of the cell that they are with difficulty distinguished from the neuro-fibrils except by special staining methods. The cell bodies of these connective-tissue cells may lie entirely within the nerve cell. The mollusk, *Helix* (Fig. 162), shows such conditions, as well as the incidental entrance of connective tissue noted above in the pediculate fishes (see Fig. 161) and in the stellate ganglion cell of *Loligo* (see Fig. 156).

Pigment normally appears in a number of nerve cells as a collection of small brown granules occupying some particular portion of the cytoplasm (Fig. 163). This is of unknown use to the cell. In disease as well as in old age the amount of this pigment is largely increased.

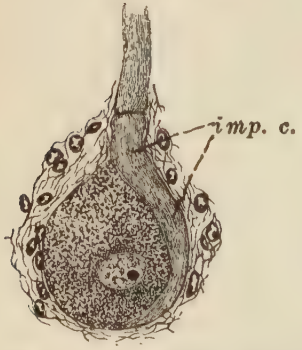

FIG. 165. — Nerve cell from the brain of a lobster, *Homarus*, showing an implantation cone (*imp.c.*) that reaches far into the cell.

A centrosphere, sometimes containing a centrosome, has been described in nerve cells by McClure, Lewis, and others. It is possible to look upon these structures either as vestigial organs left from the last division or as centers of some present kinetic operations connected with the activities of the cell. The former view seems not to be in accord with what we know of the persistence of the centrosome after cell division. And yet we shall consider it to be the best view because this structure is only occasionally found, even in the same kind of cell. Many nerve cells show protoplasmic processes which appear to unite with similar processes from other cells. This is well illustrated by the "dorsal nerve cells" from the cord of *Achirus lineatus* (Fig.

164). Part of the cell body is sometimes free of tigroid bodies and is of the same texture as the nerve fiber of which it is a direct continuation. This is called the implantation cone in the vertebrates, where it is roughly cone-shaped (see Figs. 157, 158, 160, and 161). In the arthropods it forms a long curved area which reaches around the nucleus (Fig. 165).

Technic. — The study of the nerve-cell bodies requires only carefully prepared paraffin sections, as long as this study does not extend to the processes. Staining is an important factor, and several staining methods have been evolved for the purpose of learning more about the structure of these objects. See LEE'S "Microscopist's Vade Mecum."

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THE NERVE FIBER

The nerve fiber is that part of a neuron which is specialized for conduction. It is an integral part of the nerve cell; an evagination of its cytoplasm. Its growth, or the process of its evagination from the cell, has been traced in the living embryonic development of the cell and in the regeneration of nerve tissue. As proved by experiment, it dies when separated, *in situ*, from the cell body, which then develops a new and similar process to take its place.

Looked upon in this light, we can see that its structure must be a modification of that of the cell body. This occurs in two degrees, forming two kinds of fibers, — those that retain the tigroid bodies or neurochondria of the cell body and those which do not. Some dendritic processes are the only ones that retain the neurochondria, and they do this for only a part of their course. Otherwise, the fiber is everywhere the same, a continuation of the cytoplasm of the nerve cell, containing a bundle of fibrils that are either continuations of those in the cell body or similar to them. The fibrils are parallel in a general way, and as has been stated in discussing the nerve cell, they are probably structures by whose agency the nerve impulse is forwarded through the neuron. That the fiber can forward the impulse from perceptory surface to discharging surface without the direct aid of the cell body, or even in the absence of the cell body, is proved by the experiments of Bethe on certain of the nerve cells of a crab. These cells were selected for the experiments because of the

fact that the efferent and afferent fibers both arose from a single process of the cell in such a way that the impulse paths did not lie in the main cell body at all, but passed directly from the dendrite to the neurite. This process, bearing the two fibers, was cut off and the cell body removed from the tissue without disturbing the connection between the fibers or disturbing their relations to the other tissues in any way. Under these circumstances the nerve continued to carry the impulse as before, and kept up its usual function until its death in the otherwise uninjured tissues. Its death was undoubtedly due to the cutting off of its nutrition and other trophic benefits formerly derived from the cell body with its nucleus.

This latter fact has also been observed in the pathological and experimental cutting off of masses of fibers from their nerve cells. In these cases the fibers die and new processes grow out from the same cells to take their places.

The nerve fiber is a structure that is only called into existence when either the perceptory or the motor surface of the cell is situated at a distance from the cell body. Consequently its length is variable and in some cases is reduced to the length of an ordinary cell.

The neurite, as has been previously said, leaves the cell body from an implantation cone, which is the intermediate portion between the cell body and the fiber. This structure may vary from the short conical form seen in most nerve cells to the more extensive kinds that appear in some of the invertebrate nerve cells, as, for instance, the lobster, where the implantation cone is narrowed to the diameter and continues as an extension of the fiber itself into the cell, forming a long curved path that sometimes encircles the nucleus before its substance merges with that of the cytoplasm and its fibrils are no longer to be distinguished among the neurochondria (see Fig. 165). Also see other figures under nerve cells.

There are interesting accessory tissues found in connection with the nerve fibers and used to provide them with coverings for their protection, and with support and union in their common pathways, the nerve tracts. The latter of these are the neuroglia cells, and will be treated of in the next part but one. The former are the true connective-tissue cells that form the sheaths covering most nerve fibers (Fig. 166).

These sheath cells have been thought to be of ectodermal origin and to have migrated, along with the nerve process, to the positions in which they are found. It has been proved, however, that they are true connective-tissue cells from the locality through which the nerve fiber has passed in its development. They may be compared closely with the connective-tissue cells that surround some of the nerve-cell bodies in the ganglia. A nerve fiber is not always provided with this covering. Some few kinds have none whatever other than the unspecialized connective or other tissue through which they pass. The simplest form in which a definite

covering is found is one in which a single layer of these sheath cells have united by their edges to form an unbroken tubular sheath sometimes called the neurolemma. The nuclei of these cells appear as if they lie on the outside of the sheath. They do not do this, however, as the cell-substance can be seen, with higher powers, covering the nucleus as is done in all cells.

In the higher vertebrate animals there is found on some nerve fibers, in addition to this connective-tissue sheath, another and inner covering of an entirely different nature. This is a layer of a thick oily or fatty

FIG. 166. — *A*, two non-medullated nerve fibers from a large connective in the *Octopus*. The left-hand fiber is a section. That on the right is a surface view showing the delicate, branching connective cell which covers it. Fibrillation not shown. *B*, a group of nerve fibers and parts of two other groups in the developing optic nerve of a three-months-old human embryo. × 1000.

substance called *myelin*. It forms a complete layer around parts of the fiber only (Fig. 167). As to its origin, its position between the nerve process and the surrounding sheath leaves it to be decided as to which of these two have produced it. As the neurite is a highly specialized structure which is probably expending all of its energies in the work of conduction, it is probable that the other, the sheath, is the producer of the myelin, and that it secretes it much as some other connective-tissue cells, the fat-cells, secrete the fat, by the metabolic activity of their cytoplasm.

Whether this secretion is a superficial one, leaving the myelin between the inner surface of the sheath and the outer surface of the neurite, or whether it is an intra-cellular secretion of the sheath cell in which each

cell forms the myelin in its interior, thus leaving a thin layer of cytoplasm between the myelin and the nerve process or neurite, is yet to be decided. The first view is favored by the apparent absence of any structure (cytoplasm) lying between the myelin and the neurite. The second possibility is advocated by some because of the presence of the *nodes* which separate the myelin of the two consecutive sheath cells as if it were an internal product. The usual presence of but one sheath-nucleus between any two neighboring nodes heightens this latter probability.

The myelin exists as a fine emulsion and is not homogeneous, but lies in the interstices of a fine network of a substance that resembles keratin or horn. This material gives off an odor like that of horn or feathers when it is freed from the myelin and burnt.

As has been intimated, the myelin sheath is not continuous. At various distances, long when compared with the fiber's diameter, it is constricted and separated by the substances of the sheath, thus forming the nodes. There is discussion as to whether these nodes or, indeed, the

FIG. 167. — Portion of a medullated nerve fiber from a mammal. Node shown near middle. Incisure shown near left end. × 1000.

sheath itself is to be found in those medullated fibers that pass through the brain and spinal cord of the mammals. They are probably provided with a sheath, as this appears in their development. Its apparent absence in the adult form is probably due to its extreme delicacy, there being no need of a strong and substantial sheath on the fiber in this position on account of the strong and heavy surroundings.

Where many fibers run in the same course they are usually found together, forming a nerve. This collection of fibers is held together by a connective-tissue covering, the endoneurium, and several of the smaller bundles are often found to be surrounded by a still thicker and stronger covering of the same kind of material. Such a composite bundle is also called a nerve, and its covering a perineurium.

Technic. – Fibers of all kinds may be fixed and teased and examined individually for both general features and to see the fibrillar nature of the nerve process. A fiber cannot be traced for any distance in this way, however, but must be stained by the Golgi method or the methylene-blue method. As the first of these stains can be made to have a selective action and to pick out only one or a few fibers from the great mass that exist in most of the fiber paths or nerves, it is the most useful method

known in working out the fiber details of ganglion structure. The second, or methylene-blue method is used more to follow the fiber courses near the periphery and the nerve elements in the sense organs. Both methods are capricious in their results and can be made to succeed only by constant effort and as the result of experience. One must be prepared to have them fail one time after another and yet to expect good results the next time. No one of the numerous variations will be set out at length here, but the student must have the individual direction of an instructor and work up the particular modifications that he finds most satisfactory from among the many that are described in LEE's "Microscopist's Vade Mecum."

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THE MOTOR END-ORGANS OF NERVE CELLS

By motor end-organ is meant the modification of the end of the efferent process of a nerve cell which enables it to transfer or discharge its impulse as a stimulus to another nerve cell or to a muscle cell, an electric cell, or a gland secreting cell. Such an impulse is used to discharge the secretion material or to cause it to be used *in situ*.

While differing to some degree in size and complexity among themselves, the several kinds of motor end-organs do not present the great variety of form and adaptation that the end-organs on the other or perceptory pole of the nerve cell do. Nor can we distinguish any specific cell-organs pertaining to the particular function of each of the organs as we can in the perceptory endings, especially those that are situated in the periphery.

As in the perceptory ending, the motor endings may be placed directly on the nerve-cell body or removed from it to the end of one of its processes, the efferent fiber. Like the fiber, it is an integral part of the cell, a direct continuation of the fiber itself. The exact relations of the

neuro-fibrils of the nerve cell and fiber to the motor endings are not known other than that they are direct continuations of one or more of the fibrils which become thicker and separated into a varying number of short branches that develop varicosities and an irregular outline. The terminal varicosities of the various branches sometimes attain considerable size, as, for instance, the end-organs of some electric nerves (Fig. 168). In such a case the sarcoplasm or neuroplasm probably

FIG. 168. — A nerve fiber (*nv. f.*) ending on an electroplax of *Astroscopus*. The end-plate is large and stained black with silver nitrate.

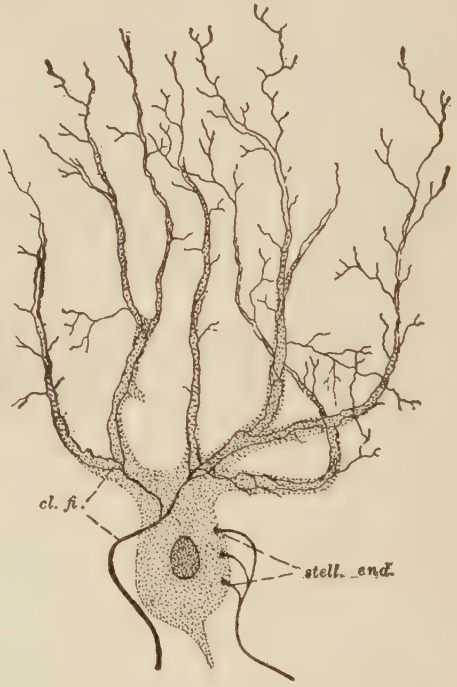

FIG. 169. — A Purkinje cell from the cerebellum of man, showing the contact of two different motor end-organs of nerve cells with its sensory surfaces. *cl. fi.*, ending of a climbing fiber on the branching dendrites; *stell.end.*, ending of a stellate fiber on a surface of the cell body. (After CAJAL; slightly modified.)

constitutes some considerable part of the structure in addition to the neuro-fibrils.

The terminal branches or telodendria of the end-organ are plainest and least irregular as well as, usually, most thickly branched in case they are used to communicate with another nerve cell.

Examples of efferent end-organs in contact with other nerve cells or their processes are most numerous. *The simplest type* of such an organ is represented by a sensory nerve cell, as, for instance, the auditory

or tactile cell of a vertebrate, without any processes and with its discharging surface directly in contact with the afferent or perceptory end-brush of a fiber leading to the central nervous system (see Fig. 198).

The most frequent type is exemplified, perhaps, by the connection established between the discharging end of a branch of a stellate nerve cell of the cerebellum with the cell body of one of the Purkinje cells in the same organ. This relation is shown in Figure 169, *stell. end.*, but its value as an example is impaired by the fact that the cell-body surface of the Purkinje cell is not its main perceptory surface, this being represented by the widely branching dendrites, which furnish us with an example of another and more typical variation of the same kind of connection as that mentioned last. This is the contact of the discharging end of a nerve fiber (here the cerebellar climbing fiber) with the perceptory processes of the Purkinje cell (Fig. 169, *cl. fi.*). The finely branched fibers of the two end-organs lie parallel with one another and in a contact that is sufficiently extensive and intimate to permit of the nerve impulse being transferred from one to the other. Thus, a neuron may have two perceptory surfaces.

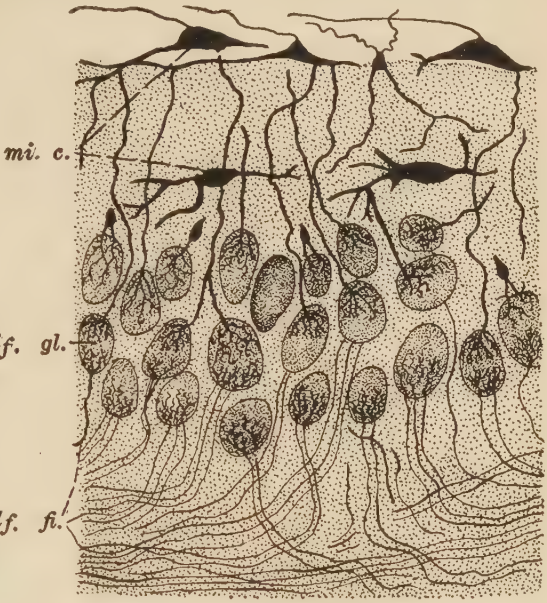

FIG. 170. — Portion of a section of the olfactory bulb of man. Stained with nitrate of silver to show the perceptory endings of mitral cells (*mi.c.*) in contact with the stimulatory end-organs of the olfactory cells, which are not shown in the figure, but whose efferent fibers enter the figure from the sides (*olf. fi.*). The oval area in which this meeting takes place is called an *olfactory glomerulus* (*olf.gl.*). (From HUBER after GOLGI and CAJAL.)

A last example of such a connection is shown by the mingling of the discharging telodendron of an olfactory nerve cell of a mammal with the perceptory branches on the end of an afferent fiber leading to one of the mitral cells of the olfactory lobe of the brain (Fig. 170).

The discharging end-organs of nerves that serve to stimulate muscle fibers into motion are well known, and two or three examples will give a clear conception of their structure. The simplest form is undoubtedly

that seen in the innervation of some of the smooth muscle fibers of the invertebrate animals. **The leech has shown such an organ** on some of the muscle cells in the circular layer of muscle tissue in its body wall (Fig.

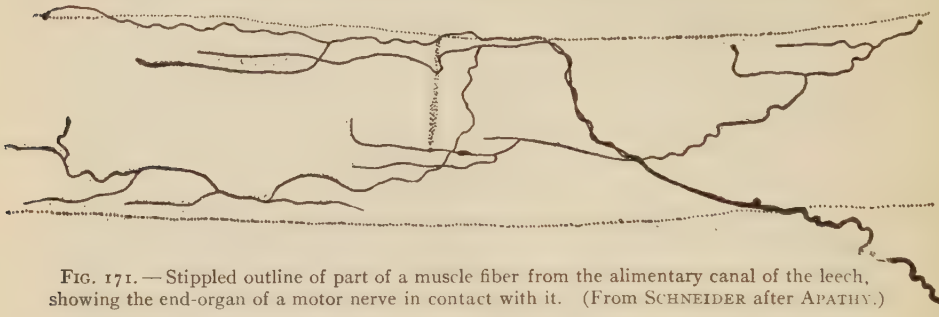

FIG. 171. — Stippled outline of part of a muscle fiber from the alimentary canal of the leech, showing the end-organ of a motor nerve in contact with it. (From SCHNEIDER after APATHY.)

171). Here the innervating fiber branches into simple and smooth divisions that apply themselves to the body of the muscle cell.

It has been questioned if these smooth terminal branches really represented the end-organs, and suggested that the real end-organs had failed to take the stain, thus remaining invisible in the preparation. Huber has found in the smooth muscle of the cat that the fibers do end in small but distinct varicosities.

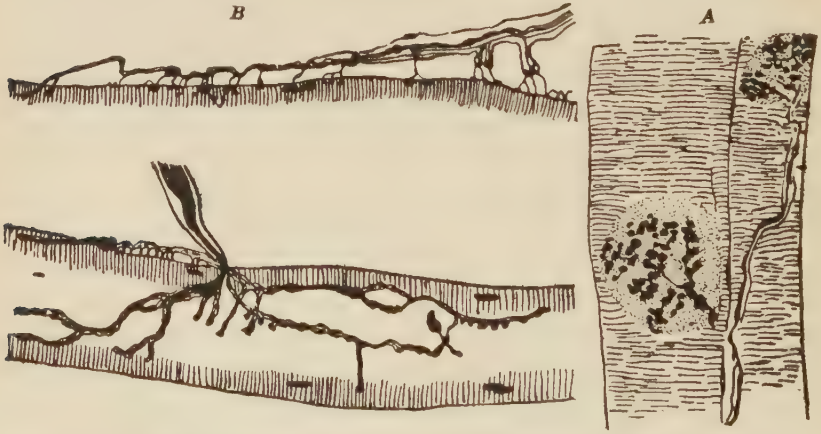

FIG. 172. — *A*, motor nerve end-organ on portions of two voluntary muscle fibers of *Lacerta*. (From BOHM and DAVIDOFF'S Histology.) *B*, motor nerve end-organs on striated muscle fibers of the frog. (After SIHLER in *Zeits. f. wiss. Zool.*)

A more specialized motor nerve-ending on muscle can be seen in the **nerve-ending on amphibian muscle tissue** (Fig. 172, *B*). The motor ending on the striated muscle fiber of the frog is branched into rather long but decidedly thick and more irregular divisions. These show, also, a feature characteristic of the more highly developed motor muscle

endings in general, the gathering of the undifferentiated muscle cytoplasm, or sarcoplasm, around the end-organ. The whole mass, partly nerve substance and partly muscle substance, is included when we use the term *end-plate*.

A further complexity and development is seen in the **motor end-organs of the reptiles** and the higher vertebrates. The lizard shows excellent examples (Fig. 172, *A*). The end branches of the nerve or telodendria are short and thick and very irregular. They anastomose to form a thick, heavy plexus. The collection of sarcoplasm around the plexus so formed is very large (see also the part on the muscle fiber). (Also read the parts on the nerve-endings on the different electric cells.)

The efferent processes of the nerve cells also end on gland cells. They thus transmit an impulse to the gland cell to secrete. Whether the impulse is necessary to the metabolism of the food materials into the secretion or whether it merely excites the cell to discharge or prepare to discharge the secretion already prepared by its cytoplasm is not definitely known. The fact that many cells secrete apparently without any nerve supply would point to the latter view in our example. In controlling the metabolism itself the nerve cell would only be doing what it probably does in muscle cells and others. One must also be careful to distinguish such endings as motor in character, because sensory endings are found in so many positions on the periphery.

The manner of ending in gland cells is well shown in the **nerve-ending in the tear gland of the rabbit** (Fig. 173). Fibers from several sources form plexes around the alveoli of this gland, and their terminal branches enter the glandular epithelium. Here they form motor endings that are pressed against the proximal and middle surfaces of the gland cells. These terminal fibers, which are the motor end-organs, differ from the fiber from which they come only in that they are irregular in contour and have several thickened portions, the varicosities, of which a number occur somewhat regularly distributed on each branch. The varicosities are about twice the diameter of the fiber and are nearly spherical in shape.

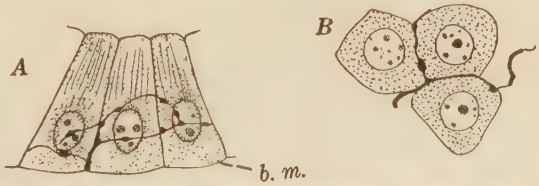

FIG. 173. — Stimulatory nerve end-organs in the epithelium of the lachrymal gland of a rabbit. *A*, lateral view, with basement membrane (*b.m.*); *B*, superficial view. × 200. (After A. DOGIEL in *Arch. f. mik. Anat.*)

These nerves are not the only ones that direct the activities of the gland. Others act indirectly through the medium of the muscle fibers that regulate the blood supply and those that act as compressors of the gland mass itself.

A widely different type of gland, the nephridium, also has motor nerve-endings which control the activities of its epithelial cells. Nerve fibers have been found which all but end in the renal cells of the earthworm. But little effort with methylene blue would suffice to bring out the terminal organ which must be closely applied to or actually entered into the cytoplasm.

FIG. 174. — Portion of renal epithelium of frog, showing stimulatory nerve-endings in the cells. (After SMIRNOW in *Zool. Anz.*)

Figure 174 shows this state as actually demonstrated in the **nerve-endings in the frog's renal epithelium.** The nerve fibrils here pass into the cytoplasm as varicose fibrils with an irregular course and in sufficient number to control the activities.

Technic. — Methylene blue is the best method for studying these structures. Nitrate of silver will sometimes give good results, but the *intra vitam* methylene-blue method will satisfy all needs when once the investigator has mastered it and adapted it to his needs.

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NEUROGLIA

The neuroglia tissue is the supporting tissue of the nervous system that has been derived from the ectodermal cells in the early history of the development of the individual. It is derived from the same layer of cells that the nerve cells themselves are, and its relations can be made clear by looking at a section of the spinal cord of a fish where the ependymal cells, which are neuroglia cells, show a condition that is halfway between a purely epithelial form and the internal neuroglia cells found in the brain, where they have assumed a form that would lead one ignorant of their origin to call them connective-tissue cells. Figure 175 represents four neuroglia cells that are graded with reference to their degree of specialization as to branching and internal position. This series is neither ontogenetic or

taxonomic, but a combination of both. It serves, however, to indicate the growth of the cell away from the surface, the moving of its nucleated body toward its internal or proximal end, and its final separation from all connection with the surface. The successive stages of branching as the cell moves away are also easily noted.

FIG. 175. — Four neuroglia cells taken from different sources to show four grades of specialization as connective cells of the nerve tissues. The specialization consists of increasing removal from the surface and the development of branches. Individual figures taken from "STOHR'S Text-book of Histology" by LEWIS.

The relation is clearer here than in any of the invertebrate tissues, where, however, the same truth holds for neuroglia as to its origin and use.

The most characteristic structural feature of neuroglia tissue is the rather small number of smooth, strong fibrils that the branching cytoplasmic processes of the cells produce. These fibrils are unlike the fibrils of simple binding connective tissue in that they are long and of a uniform size and a smooth, even contour. They do not branch as a rule, and are not produced by the cytoplasm of the central mass, but rather by the peripheral portions and its processes. The dense branching effect of the neuroglia cell, when stained by the Golgi process, is due to the staining of the protoplasm in which the fibrils lie, as well as the fibrils themselves.

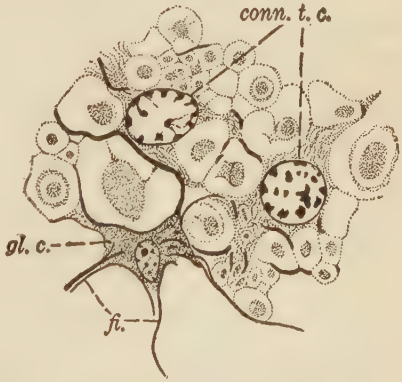

FIG. 176. — Part of a section of the spinal cord of a rabbit. *gl.c.*, neuroglia cell; *fi.*, neuroglia fibrils; *conn. t.c.*, connective tissue cell. (After K. C. SCHNEIDER.)

But few stains will properly differentiate these fibrils from the cytoplasm of the cells and from other kinds of fibers. Figure 176 shows this separation effected by means of the "Mallory" stain.

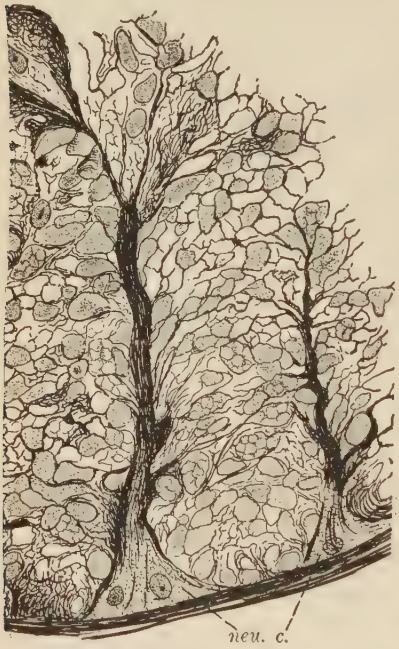

FIG. 177. — Neuroglia cells and their branches in a nerve cord of the gasteropod mollusk *Sycotypus*. *neu.c.*, neuroglia cells.

These cells must not be confounded with the real connective-tissue cells of mesodermal origin that are found in the nerve tissue. Such cells are especially prominent near the blood vessels and can easily be distinguished from neuroglia in many ways. Figure 176 shows a comparison between the two kinds. Note the finer and more numerous as well as more lightly stained fibrils of the connective tissue; also the nucleus, which is far smaller in the neuroglia cells.

In the invertebrate animals are found all stages of specialization of the neuroglia cell from the embryonic neuroblast. The fully adult forms, which will alone be shown here, usually have some portion of their body on the surface of the nerve center or nerve cord in which they lie, thus indicating their origin from the embryonic surface of the body, of which most of the nerve structures were formerly a part. Figure 177 shows a portion of a **nerve cord of a gasteropod mollusk**, *Sycotypus*, cut in transverse section. The neuroglia cells, here ependymal in form, are situated with their principal cytoplasmic masses, containing the nucleus, on the periphery, and the branching cytoplasm, containing the fibrils, extends toward the center of the nerve cord and branches freely to form a support for the numerous bundles of nerve fibers running through it.

A more involved form, of great specialization, is to be seen in the enormous **neuroglia cells found scattered in some of the nerve cords of the common leech**, *Hirudo medicinalis*. This huge cell is placed in the center of the nerve cord and sends branching processes outward to the surface. The neuroglia fibrils are contained in these processes, which produce and maintain them. The processes, here as before, act as a supporting scaffold for the nerve fibers that pass through the cord. They also flatten against the surface of the cord to which they are fastened (Fig. 178).

Although these large cells are more than probably derived from the

surface of the cord, and thus from the epithelium of the embryonic body, the exact method of their development and growth into this secondary position has not been worked out. Neuroglia cells show a strong tendency to syncytial arrangement, due, as in connective tissue, to their function.

Neuroglia is found, not only in the central nervous system and nerve cords of most animals, but also in the sensory nerve tissues on the periphery. Its most primitive form can, perhaps, be seen in the " supporting cells " of olfactory and tactile epithelia. Here the cells have no processes, and little resemble neuroglia elements, and it is only by virtue of their association with sensory nerve cells that they can be called neuroglia at all.

FIG. 178. — Transection of a nerve connective in the leech to show one of the large central neuroglia cells with its fibrillar processes reaching out in all directions to the periphery and acting as a support for the numerous nerve fibers that run between them in bundles. (After K. C. SCHNEIDER.)

In the visual tissues these cells show a distinct advance as neuroglia cells over the condition found in the other sense organs. This advance is not marked in the eyes of the lower invertebrates. A mere beginning is represented in the glia cells found in the eyes of planarian worms. In the vertebrate visual tissue, the retina, neuroglia is well developed, almost as much so as in the central nervous tissues. This is easily understood when we recall that the retina is only secondarily derived from the surface, being evaginated from the brain, which was in its turn invaginated from the dorsal surface of the young embryo. The retinal neuroglia cell, or radial fiber, as it is called, is considerably branched, and acts as an efficient support to the delicate nerve cells of the retina, from whose embryonic ancestral cells it was also derived (see Fig. 175, *B*).

Technic. — The general outlines of neuroglia cells may be most satisfactorily seen when the tissue has been prepared by one of the methods of Golgi. After the location of these kinds of cells is known, their cell bodies may be recognized in many ordinary preparations and distinguished from the ordinary connective-tissue cells in that preparation. It may be distinguished with certainty from these cells in two ways: by the use of special stains, as Mallory's stain for neuroglia, or by a study of its histogenesis. This latter way of determining the nature of the cell is most satisfactory and is not difficult.

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TISSUES OF TOUCH, OR TACTILE TISSUES

The simplest form of stimulus that can be perceived by the cells of the body is probably some form of motion, the movements of some thing, be it a solid, a fluid, or a gas. There are many kinds of such movements, from the slow pressure of a rigid mass up to the rapid motions of waves of the atmosphere or other gases. Also there are certain qualities of these movements, such as direction, duplication, repetition, and rhythm that can be perceived by some cells and not by others.

The cells which can only perceive the movements of bodies in a general way, including pressure and impact, are known as the *cells of touch* or *tactile cells*. Those which make use of the impact or pressure of special bodies under the influence of gravity or spatial relations to determine the position of the body are the *cells of equilibration* or *static cells*. Those which perceive the motion of the air waves, either directly or as transmitted and represented through the mechanical vibrations of substances affected by these waves, are called the *sense cells of hearing* or the *auditory cells*. These several kinds of nervous tissue, all alike, perceive only the *mechanical* movements of matter.

It is characteristic of these tissues that the perceptory cell itself almost never receives the stimulus first hand, but usually from some other and intervening tissue cell or dead cell, cell-product or foreign body that, while itself not sensitive, is yet able to convey the motion stimulus to the real perceptory cell by transmitting, sometimes with modifications, the movement through its own mass. We shall call such bodies the *intermediate tissues* or *substances*. These intermediate cells and materials form organic parts of the sensory apparatus, many of which would not be able to operate without them or would send in exaggerated tactile or pain sensations, or none at all.

Some few forms of tactile cells, found in various groups of animals, receive the stimulus almost directly upon their sensory surfaces and practically without the aid of intermediate cells or tissues. Such a case

is the **tactile cell**, whose afferent process terminates in a **nerve-ending in the stratified epithelium of the cornea of mammals.** It was formerly thought that the branching fibrils of this ending lay free upon the corneal surface. They probably reach almost if not quite to it. The least touch of a foreign solid practically reaches these endings directly and causes them to transmit a sensation of pain to the nerve centers. They come very near, if not entirely, to being sensory tactile endings that operate directly in response to the touch of solids and not through the agency of any intermediate cells or substance.

This form is little removed in similarity of structure and operation from the great mass of tactile perceptory organs found, in the majority of animals, at the periphery. These organs utilize the living or (less often) dead cells, among which they branch, as intermediate organs, through which they receive their motion stimuli. When the intermediate tissue is removed, by accident or otherwise, the tactile sensation is intensified to one of pain. A good example of a large and widely distributed group is to be seen in the afferent **sensory endings of nerve cells in the skin on the snout of the pig** (Fig. 179). This figure needs but little explanation, showing the end-organ fibrils passing in angular paths among the stratified epithelial cells to within two or three cells of the surface. These epithelial cells are not sensory. They can feel nothing. But the slightest touch on the surface pushes them against the nerve-endings, and the sensation is carried to the nerve centers. Nor are these epithelial cells specialized to do their work in any way that can be detected. Any other cells in this position would do just as well. In fact, many of them are dead cells.

FIG. 179. — Sensory nerve end-organ in the external epithelium of a pig's snout. (After RETZIUS.)

In the columnar cells of many animals which have no stratified epithelium in the epidermis we find a similar arrangement. **In the earthworm such a sensory structure** is found (Fig. 180). Here the sensory fibrils are placed around and between the proximal ends of the epithelial cells which convey the motion stimulus to them in exactly the same way that the stratified cells did in the pig's snout. We must realize the difficulty, however, of distinguishing these sensory endings from motor endings used to stimulate the gland cells of this epithelium into secretion.

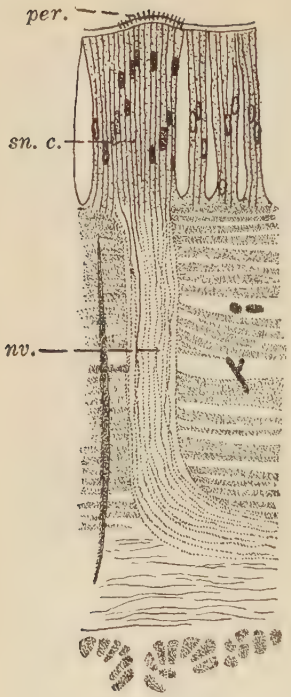

FIG. 180. — Tactile nerve-endings in the integument of the earthworm. *nv.*, nerve; *sn.c.*, sense cells; *per.*, perceptory cell-organs projecting through the cuticle. (From SCHNEIDER after R. HESSE.)

A step higher in the relations of a tactile perceptory cell to its intermediate cells is to be seen in the epithelium of the cat's foot or the Guinea pig's skin (Fig. 181). Here the sensory ending comes into contact, by cup-shaped swellings called *menisci*, with certain of the epithelial cells instead of with any of them. Those so distinguished are called, erroneously perhaps, *tactile cells*. If they can feel and transmit their sensation as an impulse to the meniscus-bearing cells, then they would be true tactile cells, and the menisci would belong to communicatory cells. As they are most probably only differentiated slightly to intensify, soften, or otherwise qualify the motion which they mechanically transmit, they are rather to be regarded as slightly specialized intermediate cells and not of any nervous function whatever.

We shall now examine a group of **tactile organs with highly developed, multicellular, intermediate tissues.** These intermediate tissues are constructed so as to transmit the pressure to the nerve-ending on the hydraulic principle that any pressure, exerted from the outside upon the walls of a closed cavity containing a fluid, will be transmitted to all inner surfaces of the walls and also to the surfaces of any objects contained therein, at a certain ratio per unit of surface.

The nerve-endings from one or more fibers, in these organs, enter the interior of a closed sac formed by several coverings of a lamellar connective tissue. Here they lie in a quantity of a fluid or semifluid substance which transmits the pressures that they are intended to perceive. We shall study two examples of this kind of structure to become acquainted with the two chief variations.

FIG. 181. — Sensory (tactile) nerve-endings among the stratified epithelial cells of a cat's toe. × 600. (After DOGIEL in *Arch. f. mik. Anat.*)

The **first of this kind of nerve-**

ending, found in the connective tissue lying between the muscles of the cat, is used apparently to record the pressures produced by the movements of the body. Each of them, known as a *cylindrical corpuscle*, consists of a very plain, almost straight and somewhat granular, single nerve termination, the end of a nerve fiber together with other tissues placed around it. Its extreme end is irregularly bent. The granular material in which it lies is called the *inner bulb* and is probably a product of the capsule cells. The connective-tissue coverings are only four or five in number, with nuclei occurring at frequent intervals between the layers. These connective-tissue cells form plate-like areas of the connective-tissue substance and the plates are joined into a series of coverings. Consequently, the appearance of the coverings is the same in any section that cuts the central axis, a series of thread-like rings of tissue with the nuclei scattered between them. The coverings are continuous with the sheath of the nerve (Fig. 182).

FIG. 182. — Nerve-endings (tactile) in intermuscular septum of cat, showing the outer capsule and the inner granular cytoplasm which contains the rod-like nerve-ending. (From BOHM and DAVIDOFF'S "Histology" by HUBER.)

Various modifications of this simple form are found, with more or less coverings and with variations in the shape of the nerve-ending, which, however, is primarily a single, approximately straight rod. Its modifications consist of granulations and fine side processes. Sometimes a second small fiber enters the capsule and forms a plexus around the main termination.

The **second specimen of a simple encapsulated nerve end-organ**, whose intermediate structures operate on the hydraulic pressure plan, is found in the skin and other parts of the periphery of mammals. The differences which it and the rest of its class exhibit, when compared with those just mentioned, are a branching and anastomosing end-organ whose fibrils are irregular in course and in shape, but whose contour is smooth instead of granular, as was the case in the preceding form. There is, also, a somewhat thinner capsule, which probably permits other sorts of motions and pressures to be transmitted than the purely hydraulic kind.

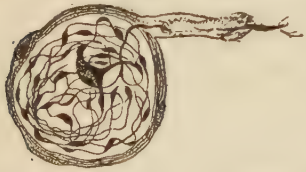

FIG. 183. — Tactile nerve-ending found in the conjunctiva of man. Methylene blue picture. (After DOGIEL in *Arch. f. mik. Anat.*)

A type of this form is shown in the **end-bulb of Krause from the conjunctiva of man** (Fig. 183). The various other forms of this group,

as the Meisner's corpuscles and the genital corpuscles, etc., differ from this specimen merely in general shape, size, and the pattern into which the terminal end fibrils are woven.

In contrast to these two forms of touch organs in which a non-cellular substance, a granular fluid, acts hydraulically upon the nerve-ending, we must note three other kinds, all of which have capsules, but in which the chief content, which is in direct contact with the nerve-ending, is a solid *cellular mass*. These are the *corpuscles of Herbst*, the *neuromuscular* and the *neurotendinous tactile organs*.

In the corpuscles of Herbst and some others that resemble it in structure, the nerve enters the melon-shaped, many-layered capsule (which is almost exactly like that of a Pacinian corpuscle) and ends in a rod much as in the cylindrical corpuscle. Instead of being surrounded by a non-cellular, fluid product of cells, however, it lies in a single layer of heavy, cubical cells which form a cylindrical covering around it. These cells are thus placed between the nerve-ending and the innermost covering of the capsule. We can find this organ, to study, in the skin on the bill of the duck and other waterfowl (Fig. 184). The inner cubical cells are of unknown function, but it is very probable that they act as modifiers of the numerous jars, rubs, touches, etc., to which the duck subjects its sensitive bill in order to learn the nature of objects by the delicate sense of touch.

FIG. 184. — Tactile (and taste?) nerve-ending in the integument of a duck's bill. Many concentric connective-tissue capsules, *fib.cap.* *cub.c.*, cubical cells surrounding the reticular end-organs of the nerve fiber. (From *Anat. Anz.* after A. DOGIEL.)

In the muscles and tendons of some vertebrates are found sensory organs composed of spindle-shaped areas of the muscle or tendon tissue itself, inclosed in a capsule and provided with the afferent fibers of sensory touch cells that enter the capsule and branch freely on the inclosed and slightly modified muscle fibers or tendon fibers within. These organs record the pressures to which the muscles and tendons are subjected during the contraction of the muscles, etc. If the pressure becomes too great, the sensation becomes one of pain. These are called **the neuromuscular and the neurotendinous organs of touch**. In both of them the method of distribution of the terminal organs is essentially the same, but also very different from the pattern formed by these organs in all the other touch structures. The fiber branches into several fibrils

which end in a number of telodendria on the surfaces of the rudimentary muscle and tendon fibers (Fig. 185).

FIG. 185. — Neurotendinous nerve end-organ in the rabbit. (From HUBER and DE WITT in *Journ. of Comp. Neurology.*)

All the above organs were found placed in the soft tissues of animals, and no hard parts were developed in connection with them. It is true that the cuticle of the earthworm intervened between the source of the motion and the simple epithelium cells that more directly acted to pass the movement on to the nerve-ending, but it cannot be said that this cuticle was developed in any way to perform this as a duty. The two structures to be demonstrated now will each show such a rigid organ, in the one case a cuticle which is a cell-product, and in the other a rigid hair made from the dead and hardened bodies of the cells themselves, which is developed solely to act as an intermediate structure in transmitting the motion stimulus. These are **the tactile hairs of the crustacean**, *Palæmonetes*, **and the tactile hairs or vibrissæ of the cat and other mammals.**

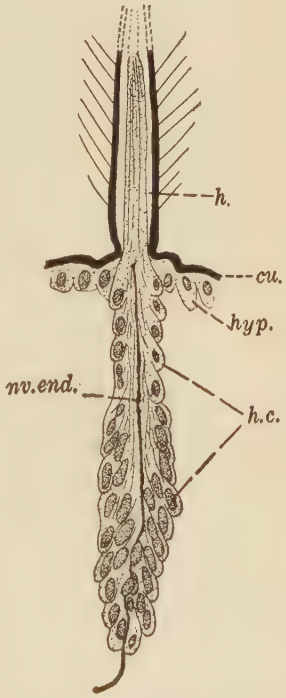

FIG. 186. — Tactile end-organ of a nerve fiber in the tactile hair of a shrimp, *Palæmonetes*. *cu.*, cuticle; *hyp.*, hypodermis, which is invaginated at (*h.c.*) into the hair cells. *h.*, outer structure of the hair; *nv.end.*, nerve-ending in the lower part of the hair. (After PRENTISS.)

The crustacean in question has much the same sort of integument that the earthworm had, — a layer of simple columnar epithelial cells that produce a cuticle. One of the chief differences is that the cuticle is thicker and, in addition, is usually impregnated with salts of lime. This makes it much harder and more resistant to the perception of outside movements through its boundaries. The sensory nerve supply comes to the periphery as it did in the earthworm, and could receive the tactile stimuli much as the earthworm's nerve-endings did were it not for the thick and hard shell. A simple modification of the whole

neighboring region takes place, where each tactile ending comes to the periphery, so that the slightest stimulus can be received. The simple epithelium at such a point is invaginated into a group of hair cells (Fig. 186, *h. c.*), whose distal ends are lengthened out so as to make a long, thin, hairlike projection (*h.*) from the surface of the body. These cells form a cuticle, as do all other epithelial cells, but it is not as thick as that on the rest of the body, and on account of the form of the cells from which it is derived it is shaped like a hair. The nerve-ending is single and large. It probably represents the ending of a single cell, and not one of the numerous branches, as do the endings of like nature in the pig's snout. It lies in a position homologous to that occupied by the tactile endings in the other epithelia at the bases of the epithelial cells. It extends up into the fiber in this position for a considerable distance, and the latter part of its course must represent a passage between the sides of the cells, while the earlier part was undoubtedly a contact with their bases.

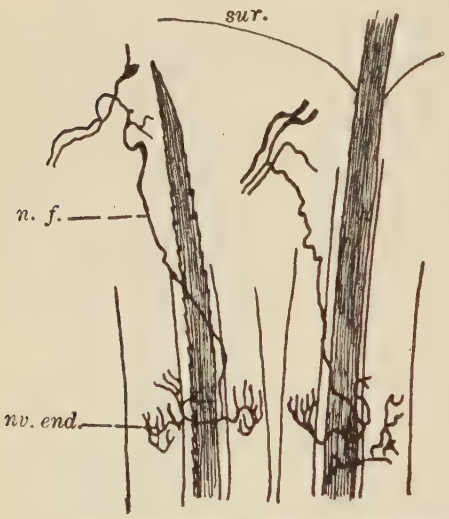

FIG. 187. - Tactile, sensory nerve-endings placed on the bases of two mammal hairs. *sur.*, surface of skin; *n.f.*, nerve fiber which ends in the basket-work end-organs at *nv.end.* (From EDINGER and HALL, after VAN GEHUCHTEN.)

The second example is that of the mammal's tactile hair. For the structure of the hair itself, see the part devoted to this subject in Chapter XX. The nerve supply consists of the terminal branches of a single fiber which approaches the hair follicle and divides into two branches that encircle it just below the mouths of the sebaceous glands. This ring gives off branches that are naked and varicose and run a short distance distally before terminating. There are a considerable number of these end fibrils which lie outside the glassy layer of the follicle (Fig. 187).

The impulse in this case is transmitted in almost exactly the same way as in the crustacean's tactile hair, although the two hairs are so differently formed. In both the hair acts as a lever, transmitting the slightest contacts with its outer portions, as greatly intensified motion stimuli to the nerve end-organs at its base. In one case the nerve termination is inside the hair, while in the other it is outside. Some mol-

lusks, as *Chiton*, have analogous organs of touch on their shell-covered surfaces.

One other perceptory power must be considered here, and that is the perception of heat and cold. Certain parts of the body surface in man perceive very small differences of temperature, while others can only feel much greater changes. When great extremes are properly applied, they give the same result, a sense of intense cold, which means a destruction of the organ and consequently of the power to perceive any heat.

These sensory endings have not been discovered histologically. It is possibly true that they are some of the same end-organs that also perceive contact or other tactile stimuli. Or they may be specialized to perform the thermo-perceptory function alone. It might prove possible to discover them by a process of comparison and elimination in the various regions that possess them or do not possess them.

Technic. — The ordinary sectioning and staining methods are of no value in the study of these tissues. Silver nitrate gives some good pictures of the structure, but the methylene-blue method is the principal means by which we have attained our present knowledge of their structure. This method, we must repeat, is not one that cannot be learned out of a book. The instructor must help and advise the student, and they must adapt one of the numerous forms of this method, as set out in Lee, to their needs.

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THE TISSUES OF EQUILIBRATION OR STATIC TISSUES

The static tissues or tissues of equilibration are tissues that record the position of the body or some larger part of it with regard to gravity or to the body's successive positions in space. It is impossible, sometimes, to differentiate between these two forms of the function; while in other examples, very good evidence has been obtained to separate them. These tissues appear as an epithelium.

As has been said, this function must be looked upon as a very delicate form of touch whose tissues are so placed as to perceive and record the positions of small and heavy bodies which press or strike against them by gravity, or, by inertia when the organism moves and changes its position. The flow of fluids over the sensitive surface of some of the

static cells also tells the animal when its body has moved or turned in certain directions.

With a few exceptions (the Crustacea), and on account of the great delicacy of the stimulus, the static cells, unlike the coarser tactile cells, receive the impression *directly* upon their perceptory end-organ. This would mean that they possess no intermediate tissues unless we may consider the crystals of lime, chitin, and foreign matter, that are operated on them by gravity or inertia, as intermediate tissues. Since these carbonate of lime concretions, chitinous plates, and even foreign bodies, as grains of sand or streams of fluid are necessary, and often organic, parts of the apparatus, we shall designate them as the *intermediate substances*.

On account of the delicate nature of the tissues and their operations, the surface that contains them is usually invaginated into some sac or follicle inside the body. This leaves a mouth or duct leading to the exterior and remaining open in primitive forms of the organ, but closed and cut off entirely in the more specialized forms.

The perceptory end-organs of the static nerve cell, which are rod-like or hair-like processes, are placed directly upon the cell body, which is a part of the surface of the static epithelium. The only exception to this is the same case mentioned above as an exception, and it will well serve as our first subject of study, **the static organs of the Crustacea**.

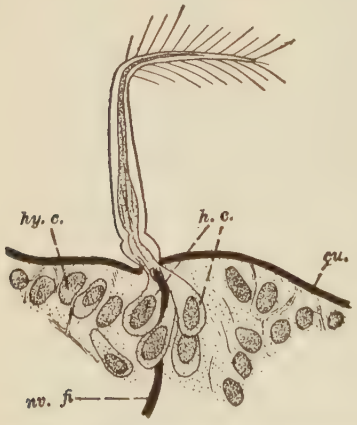

FIG. 188. — A static sensory hair from the statocyst of the shrimp, *Palæmonetes*. Formed similarly to the tactile hair (Fig. 186) but with a thinner cuticle on the hair; *cu.*, cuticle; *hy.c.*, hypodermal cells; *h.c.*, hair cells; *nv.fi.*, nerve fiber. (After PRENTISS.)

These structures will show us better than any others the relation of the static organs to the tactile organs. In the prawn, *Palæmonetes*, the static organ consists of an invaginated hollow or chamber in the side of a joint of the claw. This chamber remains in communication with the exterior by means of its opening at the point of invagination.

Inside of this pocket we find some particularly delicate "touch hairs." That is to say, they are formed like touch hairs in general, except that they have finer and more numerous ends and that they are bent so as to have their sides most accessible to anything that may touch it (Fig. 188). These hairs are situated in groups on various parts of the interior surface of the sac and are played upon by grains of sand, etc., which the shrimp

manages to insert in the sac for that purpose. The animal is conscious, when a hair is touched by the sand grain, of which part of the sac the hair was in and is able to guide its movements accordingly. By "conscious" is not meant the same thing that the word would imply in a mammal, but a correlation of the particular hairs touched with the use of certain muscles to maintain the animal in an upright position with regard to gravity. Compare Figure 188 with the drawing of the tactile hair shown in Figure 186 and see how essentially alike the two are.

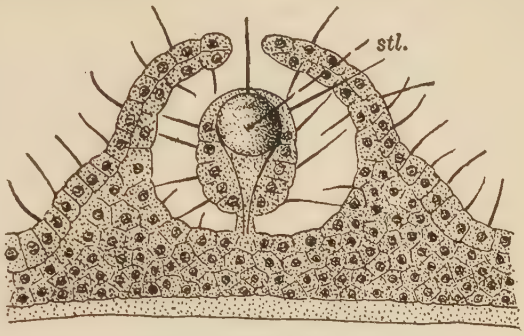

FIG. 189. — Tentaculocyst (statocyst) of a medusa, *Rhopalonema*. *stl.*, statolith inclosed in a pedicle which sways with the animal's motion and records its movements by the hairs that project from its surface. (From LANG after HERTWIG.)

Whether the action of the sand particles or *statoliths* upon the static hairs, in this creature, will convey to the nerve centers an accurate measure of spatial movement or not is not known.

An example of an organ probably used to determine the direction of movements of the body in space is to be seen in some of the **static organs of medusæ**. In these forms a body surface is invaginated into a more or less complete sac, and from the lower wall of this sac a pedicle arises, containing in its tissue a crystal or concretion of lime, or sometimes several of them (Fig. 189).

The cells lining the walls of both the cavity and the pedicle, which is called a *tentaculocyst*, are provided with sensory hairs, and the least motion of the body must convey a record of action by the touching of the sensory hairs on one side or the other of the tentaculocyst and cavity. While gravity probably makes some record of its pull, the waving of the body edge to and fro in swimming must cause a much greater stimulus, and the organ therefore records the motions of the body in space by the inertia of its heavy statoliths.

Auditory cells have been described as formed on the walls of these cysts. While admitting the possibility of this, the writers do not think that the cells described can be used to hear sound.

A beautiful example of a statocyst, used to orient a part of the body, is to be seen in the *organ that occurs in the "foot" of some plecypod mollusks*. Figure 190 shows a picture drawn from a section of **the statocyst in a small plecypod**, *Cyclas*, a fresh-water form. The cavity of this cyst, which was invaginated and cut off from the ectoderm, is lined with an

epithelium that also consists of two kinds of cells. The sensory cells, bearing each from 60 to 100 long, stiff sensory hairs, lie apart and touch each other only by the edges of their flange-like upper surfaces. Between them, and wedged up almost in a line with them, lie the large supporting cells which seem to be, in this case, of connective-tissue origin on account of their not touching the epithelial surface. An embryological study of these cells would determine their origin. Lying in the cavity, and just clear of the long sensory hairs, is the round statolith which has an organic basis, probably of chitin, that is impregnated with car-

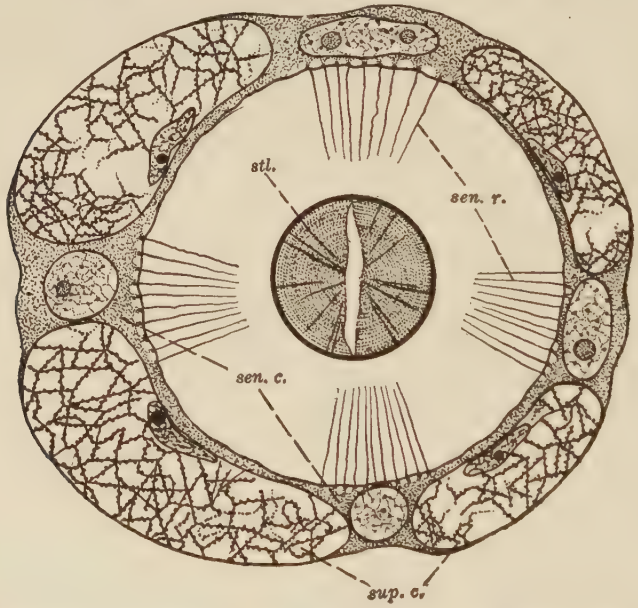

FIG. 190. — Statocyst of a species of *Cyclas*. *stl.*, statolith; *sen.c.*, sensory cells; *sup.c.*, supporting cells; *sen.r.*, sensory rods.

bonate of lime. The pressure and impact of this statolith on one or the other of the groups of sensory hairs must tell exactly in what position the foot is lying, and, consequently, which way it is to be moved next. The foot moves entirely independently of the position of the rest of the body. Watch a *Unio*, or better, an *Ensatella*, that has been dug up and left on the wet sand. Turn it in different positions and see how its foot always attempts to go *down*.

This organ should be studied in the living embryos and young of *Cyclas*. Here it is most easily observed, and the statocyst is seen to be in constant, gentle motion. We can therefore conclude that it stimulates the hairs by a rhythmic impact rather than by a pressure or single impact.

Various forms of this same organ occur through the mollusk series,

the highest development being reached, probably, in the **statocysts of the active cephalopods** where the two organs are much enlarged and the epithelium is much differentiated. It is possible that part of it has an auditory function in the squid. This animal has its two "otocysts" (we shall hereafter term them "*statocysts*") enlarged into spaces of some size and considerable differentiation as to shape. Several bars of the surrounding capsule of cartilage project into this space; and the epithelium which lines it, while it is thin and undifferentiated in most regions, has several strongly specialized portions. The use of these highly differentiated portions can be partly inferred from the habits of the animal. No creature has better control of its swift movements and rapid changes of position, whether swimming in schools at sea or in following the twisting and turning of its agile prey, the mackerel, and other fish. It is probable, therefore, that the best-developed sensory epithelium of this statocyst is used to record and so control its motion. It is improbable, although possible, that the statocyst has some auditory function to perform.

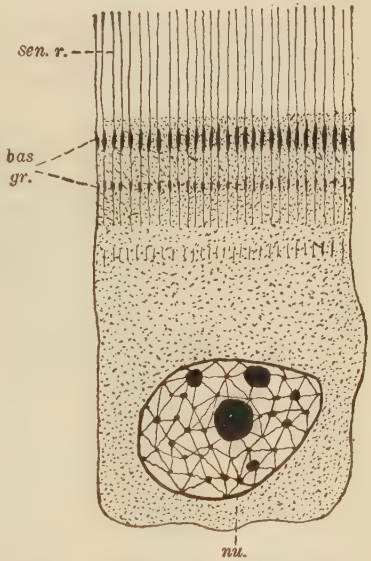

FIG. 191. — Static sensory cell from the statocyst of a squid, *Loligo Pealii*. *nu.*, nucleus; *sen.r.*, sensory rods; *bas.gr.*, basal granules of two grades. (From a drawing by A. F. McCLINTOCK and E. W. BIXBY.)

The most specialized of the lining epithelium is found on the median posterior side of the sac. Here the cells have formed several layers, the most distal of which lie in several rows and are provided with numerous rods which resemble cilia in appearance. As all other classes of mollusks have moving cilia in this position, it is possible that these rods move and are cilia, or at least are modified cilia. The intra-cellular portions of these rods are furnished, inside the cell, with several rows of spindle-shaped enlargements or knobs known as *blephroplasts* (Fig. 191). The sensory cells lie among more numerous sustentacular cells.

A layer of cells containing ganglion cells lies beneath these perceptory cells and separates them from the underlying capsule of cartilage.

The insects undoubtedly have great static powers. — Their life and actions show this. And yet no great specialization of any tissue for this purpose has been found. The only case in which such an organ has been surmised is in the *Diptera* or flies. These animals have the rudi-

ments of the second pair of wings developed into the so-called "*balancers*." The writers have found by experiment, however, that flies can fly as well without these organs as with them. Insects certainly cannot use sight to maintain their static equilibrium. Most of them cannot see far enough or well enough to do this. And even when blinded or in darkness, the static sense is not gone. Their activities would also demand a spatial sense, and yet evidences of structures used for this purpose have not been found.

The static tissues of the vertebrates have been complicated by the specialization of parts of their static epithelia to hear sound, as is de-

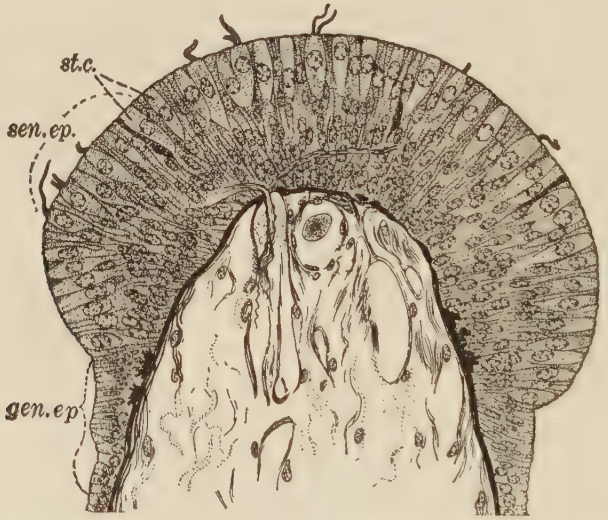

FIG. 192. — Part of a longitudinal section of an ampulla from the skate, *Raja lævis*, showing a transverse section of the sensory epithelium on the medium septum. *gen.ep.*, general epithelium composed of static cells (*st.c.*) and supporting cells. *sen. ep.*, sensory epithelium. A nerve fiber can be seen entering the epithelium and dividing.

scribed in another part of this chapter. We shall study the sensory epithelium found in the ampullæ of the semicircular canals as probable examples of tissues which perceive the *spatial* movements of the body, or of the head when that part is moved independently. The origin and general relations of the semicircular canals have been indicated in another part. We may repeat here that they are integral parts of the statocyst cavity and that one end of each, where it joins the utriculus, is enlarged in diameter to form an *ampulla* of which there are three, one for each canal. This ampulla contains an oval cavity, in the fish which our figure represents, and the lining epithelium covers the entire interior of this cavity, including a *ridge* rising across its middle at right angles to the length of the lumen. This ridge rises almost exactly halfway up,

thus cutting the lumen down to one half its diameter at that point, which is the widest point in the ampulla, and consequently in the whole length of the tube.

The **sensory cells are placed on the edge of this ridge**, a transverse section of which, from *Raja lævis*, is represented in Figure 192, also an enlarged figure of the same structure in *Amieurus* by Figure 193. These cells are rather large, and do not reach down to the basement membrane, being supported by contact with supporting or sustentacular cells whose proximal ends rest by broadened bases on a well-developed membrane.

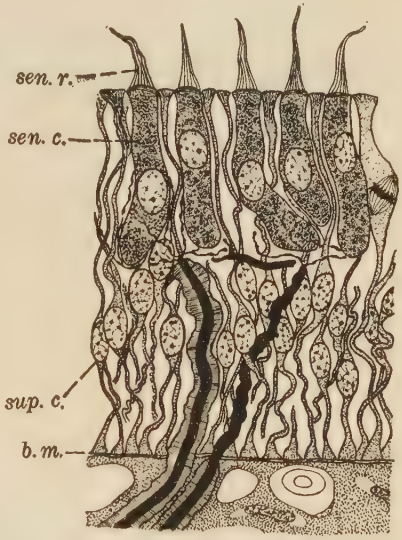

FIG. 193.—Sensory epithelium from median septum of ampulla of catfish, *Amieuris catus*. *sen.c.*, sensory cells; *sen.r.*, sensory rods; *sup.c.*, supporting cells, one of which shows mitosis; *b.m.*, basement membrane through which two nerve fibers pass. One fiber is naked while the other carries its medullary sheath into the epithelium. They both ramify as nerve-endings on the bases of the sensory cells.

The sensory cells have, as cell-organs of perception, peculiar filaments projecting from their distal surfaces. These filaments (Fig. 193) are grouped into a single projection which is very delicate and is partly embedded in a gelatinous coating which covers the ridge. The nuclei are placed about halfway in the height of the cell and are oval, with a distinctive chromatin pattern. They are larger and clearer than the nuclei of the sustentacular cells. These latter cells have the nucleus a little lower than the middle and entirely below the row of static nuclei.

Medullated nerve fibers enter the connective tissue of the ridge freely and extend up in all directions to the epithelium. Passing through the basement membrane, they usually lose their medullary sheath and divide into smaller fibril-bundles or into single fibrils to innervate the sensory cells, receiving from them an impulse when they are stimulated.

This stimulation of the perceptory cells is probably performed by currents of the fluid which fills all parts of the statocyst, including the canals and their ampullæ. When the body turns in any direction, the fluids in such of the three canals as lie in or at a sharp angle to the plane of motion pass backward or forward through the canal by their inertia, and, flowing over the ridge so well placed in their path, stimulate the sensitive cells on its edge by causing their delicate processes to bend and

vibrate in the current. This fluid thus acts as the intermediate substance.

It is possible that the purely static function with reference to gravity is performed by other sensory areas in the larger cavities of the sacculus. Here, particles of lime act as the intermediate substance instead of a fluid, as in the ampulla, or a cuticular structure, as in the cochlea. Figure 194 represents a nitrate of silver preparation from the mouse to show the nerve distribution in such a sensory region of the ventriculus. This region is known as a *macular acustica*, and its cells greatly resemble those of the ampulla ridge.

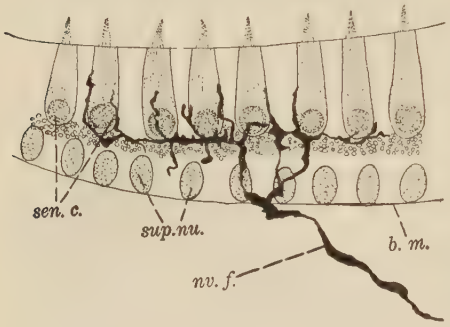

FIG. 194. — Portion of *macula acustica sacculi* of mouse, treated by Golgi's method to show nerve-ending on sensory cells (*sen.c.*). *b.m.*, basement membrane; *sup.nu.*, nuclei supporting cells; *nv.f.*, nerve fiber. (After VON LENHOSSEK.)

Technic. — To have a complete understanding of a static organ, it is necessary to have a great variety of preparations. The finer anatomical relations must be studied, and these can best be got, in most cases, by well-prepared serial sections. Such series are difficult to prepare on account of the heterogeneous tissues that go to form these organs. In the cases of the higher animals, various bones, cartilages, and otoliths have to be dealt with as well as many grades of connective tissues. A decalcifying fixative should be used in these cases, and care should be taken not to render the remaining connective tissues unduly hard and brittle. Zenker's fluid and chrom-aceto-formal were very successfully used, sometimes followed by a double embedding in paraffin and celloidin. When it was desired to stain before sectioning, a saturated solution of sublimate with 5 per cent of acetic acid was used to fix. Silver and methylene blue are essential in making a study of the nerve elements. The writers also found that carefully teased specimens that had been macerated somewhat were invaluable in class demonstrations. This latter method was modified as follows to form a valuable process in the study of any epithelia. The tissue was first placed in a macerating medium that was at the same time a fairly good fixative; weak osmic and chromic acids, as ordinarily used to macerate, were found to be the best, and one third alcohol also gave good results. The tissue was handled with the greatest caution, and somewhat before it was macerated enough to tease, it was washed, stained, dehydrated, and embedded in paraffin. Sections of rather greater thickness than usual were then

cut and laid on a slide, and xylol was used to remove the paraffin. The dropping of a small amount of balsam was now enough to slightly separate the cells, and this was further brought about by the placing of the cover glass in position. If the displacement of the cells was too great, a little collodion and clove-oil mixture was first placed on the glass, and this served to retard the separation, which could also be controlled in many other ways. The result is perfect in all ways but one; *i.e.* the tissue having been cut in sections makes it impossible to say if each cell in the finished specimen is whole or not.

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THE TISSUES OF HEARING OR AUDITORY TISSUES

These tissues are also modified forms of tactile tissues. They are more delicate refinements, even, than the static tissues, since they perceive and record the finest differences in the rhythm of successive impacts caused by the waves of the atmosphere that are known as sound.

The intermediate tissues in this case consist of two kinds. One kind is intended to collect and thus intensify the sound and to convey it to the auditory tissues which are usually very internal. These are known as tympana and pinnæ. A second kind are the delicate intermediate structures that directly apply the sound waves to the auditory nerve cells. These latter must be of exactly the right texture to properly operate upon the delicate and highly specialized perceptory nerve-endings, which here consist of various rods, hairs, or plates. In some forms, these intermediate substances do not occur. Where they do occur, they are special cell-products.

In the vertebrates the auditory tissues are specializations of the static tissues, and therefore the two are found to be closely related and parts of the same organ. This is not the case in some of the insects where there is a separate origin and position.

For us to determine whether a given sense organ is auditory or static in function is sometimes a difficult task, when we investigate such organs as are other than our own. We must consider various points in this connection. A comparison of the tissues of the mammals with our own, structurally, tells us that most mammals must hear. It also tells us

that birds probably hear. Its value in the case of other vertebrates is negative, while for all other creatures it is entirely valueless. The presence of sound-making apparatuses in the animal is rather poor evidence that an auditory power is also present. Many animals are practically mute, and yet have the keenest of ears for the sounds made by enemies.

The voice of insects is often put forth as evidence that they must hear. The plecypod mollusk, living on the bottom of the stream where the current pouring over stones and sand must make a noise, might use the otocyst to hear the noise. But the tiny *Cyclas*, living deep in the mud and ooze of the stillest ponds, has really no possible use for its otocyst other than to know which direction is up and which is down. Most mollusks probably hear nothing.

The presence of accessory tissues to gather and transmit the sound is good evidence. The tympana and pinnæ of various kinds tell unmistakably that an auditory function is at least a part of the organ's duties. This is the determining factor in the frog, where experiment is uncertain. Experiment is difficult, but some of its positive results are conclusive. The only reaction that we can trust is a sudden motion or start when the sound is made, and it is possible that many forms would not move even if they heard the noise. A too loud sound might also stimulate other sense organs.

We shall study four forms of tissue that can undoubtedly perceive sound: the auditory hairs of the mosquito and other insects; the chordotonal organs of an insect; the ear of an insect, and the ear of a Guinea pig, which much resembles that of man (for the possible auditory organ of the cephalopod mollusks, see the part on equilibration).

The hair-like auditory organs have been best studied in the antennæ of the male mosquito and in the auditory hairs of some larvæ (*Corethra*). The mosquito (a male) was fastened to a glass slide by the feet so that he was living and in health, but quiet enough to be put under the microscope and studied. Tuning forks were then sounded in a succession of strong notes of various pitches, and it was observed that at certain notes the hairs vibrated, strongest at 512 vibrations per second and weaker at some adjacent notes and in some of the other hairs. The vibrations of these hairs act upon a very peculiar and complex organ found in the second basal segment of the antenna. A nerve carries the stimulus from this organ to the brain. As this organ only acted when the hairs were at the proper angle to the sound waves, and as this angle extended from directly in front for some distance toward the outer side of each antenna, it can be seen that the male mosquito can perceive both the sound and its direction, and thus can find the female in the dark.

The histology of this organ is, in principle, like that of the tactile

hairs of many arthropods, and also closely resembles the static hairs of the shrimp, which we have studied, thus showing the close relationship that exists between these three forms of sense organs (Fig. 195).

FIG. 195. — Longitudinal section of second antennal segment of a mosquito, *Mochlonyx culiciformis*. *aud. or.*, auditory organ. (After CHILD in *Zeitschrift f. wiss. Zool.*)

The chordotonal organs in the limbs of some katydids represent another different and somewhat more specialized form of auditory apparatus. It is a wonderful organ because of the many kinds of highly differentiated tissues that coöperate to form it. A very large trachea comes into the limb and lies in very close contact with one side of it. The outer cuticle of the limb becomes thin on an oval area of this contact, and this area forms the tympanum of the auditory organ. The tympanum is thus made of two thin layers of cuticle (for the trachea is a portion of invaginated integument) between which lie the two layers of simple epithelium which have formed them.

In the anterior of the two widening spaces, where these two walls of the tympanum separate, lie the auditory, perceptory cells (Fig. 196). They are of two kinds, in which the differences are mostly those of size and general form. It is also possible, if these perceptory cells were derived from the ectodermal tissues, as they probably were, that the cells of one group originated from the tracheal epithelium, while the others came from that of the outer integument of the limb. These cells are connected with some central ganglion by two nerves that unite upon

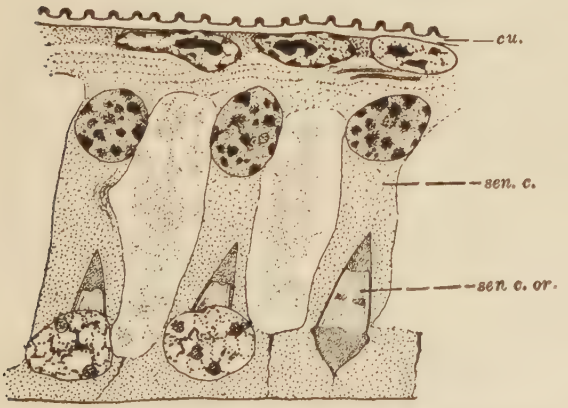

FIG. 196. — Part of a longitudinal, vertical section of the fore tibia of a young katydid, *Microcentrum laurifolium*, 10 mm. long. Chordotonal organ. *sen.c.*, sensory cells; *sen.c.or.*, cell-organ of sound perception; *cu.*, cuticle of trachea. × 1200.

leaving the limb. The peculiar structure of the cell is remarkable, and is seen only in the auditory organs of insects. Its cell body is found in the ganglion (Siebold's or the supra-tympanal ganglion in this case), and the efferent processes from several of these cells form the nerve fibers that pass into the body. The afferent pole is elongated into a moderately long fiber, on the end of which is seen the huge auditory end-organ of the cell which is often larger than the cell body itself. Its interior is occupied by space drawn out in the axis of the fiber and lined with a cuticular shell that is open on the end toward the cell body, but closed distally and thickened into a conical mass. The central portion of the afferent fiber coming from the cell is directed into the open end of this peculiar end-organ, and ends as a nerve fibril that projects freely into the body. Its free portion in the cavity is called the *axial filament*, and its vibrations are caused by the sound waves working through the tympanum as a medium, or, perhaps, directly. These vibrations produce the stimulation of the nerve cell. The distal portion of this sensory cell-organ is further extended to form a means of attachment for the cell to the tympanal surface. This is called the *terminal filament*. The presence of other nuclei in the scolophores of some insects might lead one to believe that the whole apparatus was not unicellular, and that other cells than the ganglion cell had taken part in the formation of the scolophore. The axial filament at least is a part of this cell. When no special tympanum exists, as in some lower larvæ (see below), the terminal filament may act as a tympanum itself.

As has been said, there are two groups of these cells. One is known as Siebold's ganglion, and its end-organs are attached to the trachea. They form a long row of cells of diminishing length, and probably each cell is adapted to respond to a note of different wave length. The other group is known as the supra-tympanal ganglion, and its end-organs are attached to the outer walls of the limb. Their exact function would make an interesting experimental study.

A somewhat simpler organ of the same kind exists in the limbs and body walls of many other insects. The tympanum may be entirely lacking, and yet the chordotonal organ be fairly well developed. This condition is usually accompanied by a lack of voice in the species, and gives rise to some doubts as to whether the creature can hear or not. If it cannot hear, we must then decide as to whether or not the organ is degenerate, or a rudiment of one that will be used to hear later in the history of the race.

The apparently simplest form of the chordotonal organ is found in the body tissues of many insect larvæ. In this form, as exemplified by the structure of the larva of a fly, *Chironomus*, the auditory cells occur in many segments of the body in small groups of from one to three or more.

The terminal filament is long and slender, and is attached to some part of the body cuticle. This brings a tension upon the central ganglion. In many cases a *ligament* is developed leading from another part of the body wall and attached to the ganglion cell mass. The short length of this ligament brings the tension between two parts of the body wall in the same segment and relieves the delicate nerve and ganglion, as well as the central ganglion, of nearly all strain. At the same time it allows the ligament-filament cord to vibrate under tension, and thus stimulate the axial filament which lies in the scolophore in the middle section of the compound tympanic cord (Fig. 197).

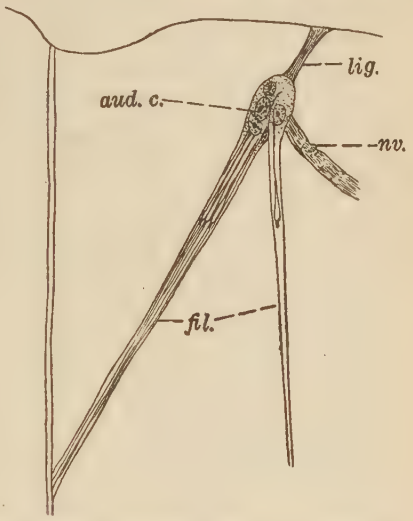

FIG. 197. - Chordotonal organ of a *Chironomus* larva. *nv.*, nerve; *lig.*, ligament; *fil.*, "terminal filament" or cell-organ of sound perception; *aud.c.*, auditory cells. (After GRABER in *Arch. f. mik. Anat.*)

On account of its lack of an internal air space and an externally differentiated tympanum, we must look upon this last auditory organ as the simplest insect form, especially that one which lacks a ligament.

One more insect form should be briefly examined, as exhibiting the most highly specialized form of the insect's auditory organ. This is the "ear" of the "grasshopper" or locust. This organ consists essentially of the same auditory cells, with their terminal filaments resting against a very large tympanum. The tympanum is stretched across the enlarged opening of an abdominal trachea that it closes, with the exception of a small pore left to allow of an equal distribution of air pressure. This allows the membrane to vibrate freely. Resting against the tympanum, and attached by their terminal filaments to two horny irregularities on its surface, are the auditory nerve cells or scolophores. The nerve, which comes from the ganglion formed by their assembled cell bodies, passes over the inner surface of the tympanum and into the body, where it enters a central ganglion.

A strong peculiarity of all the above insect auditory organs is the small number and high specialization of the auditory nerve cells or scolophores, which, with the possible exception of the mosquitoes, are found in all of them. In the mosquito the perceptory nerve cells are found in the second basal joint of the antenna, where they form a peculiar ganglion (see Fig. 195).

Most of the vertebrate animals have a sense of hearing which is made possible by the possession of auditory sense cells and all the accessory tissues necessary to gather and intensify the sound waves and transmit them to the perceptory cells.

The perceptory cells in this case have been developed or evolved from the static epithelium or from a common epithelium from which both of these have originated. In the few other forms of animals that can hear, the auditory tissues have originated from entirely different parts of the body.

The process of the evolution of auditory cells from the tactile or static epithelium of the internal ear-sac is one whose progressive steps can apparently be traced in the taxonomic series of vertebrates. Fishes apparently are just coming into their power of hearing, and it is a question if they can hear or not. Some probably can hear a few low sounds. From the fishes up, the series of amphibia, reptiles, birds, and mammals show successively higher stages in the development of a part of the ear-sac into a *cochlea* or region of auditory perception, until, when we arrive at the mammals, we find the beautifully arranged series of auditory cells and accessory supporting cells which are grouped in a row which winds in a spiral to save space. Like a snail shell, this hollow, spiral, tubular part of the sacculus diminishes in size, and the resulting different lengths of cells probably perceive lower or higher notes of sound.

The auditory epithelium of the Guinea pig will serve as an example, and we shall study the structure, although we cannot hope to entirely understand the mechanism. Only the membranous portion, and especially its specific epithelium, will be treated of in this description, and we shall begin by short embryological explanation.

The whole epithelium under consideration was originally a part of the body epithelium on the sides of the head. At an early period (10 days in the rabbit) this epithelium thickened and was invaginated into a sac with a narrow duct connecting it with the exterior.

The sac continued to enlarge and the duct to close until it was cut off entirely and obliterated. This left the sac as an internal cavity lined with an epithelium. The sac enlarged and constricted in the middle until it formed two sacs united by a duct. These two compartments are called *utriculus* and *sacculus*, while the duct is known as the *utriculo-saccular* duct in the adult.

The utriculus now evaginated from its sides the three semicircular canals, three curved tubes opening into the sac with both ends. Each tube was enlarged into an ampulla which is described under the static tissues. The epithelium of different parts of both sacculus and utriculus were differentiated in several regions, while the whole complicated organ was encased in a bony covering which formed around it.

Meanwhile the sacculus had evaginated, from its inner epithelial surface, a long tube which has curled into the snail-shell-shaped structure mentioned above. This is the *cochlea*, whose rudiment is to be seen as a small evagination from the inner surface of the sacculus in the lower vertebrates, which has become elongated for some distance by continued invagination in the birds, where it is straight or partly curved, and which is thus curled up in the mammals on account of its great length and development.

This spiral, membranous tube does not retain a round shape, nor does it fill the rounded bony cavity which is provided for it in the Guinea pig. As is best seen in a transverse section of one of its coils, it is applied by a third of its circumference to a narrow area of the outer wall of this bony case, and is met by a bony and muscular septum or shelf that reaches out from the inner wall to form a contact with a second third, which thus lies at about right angles to the first mentioned. The last third of the epithelium-bearing, membranous tube is stretched through the cavity as a septum which divides the triangular interior of the membranous tube from the large part of the bony tube which the membranous cochlea occupies.

In section, then, the membranous cochlea is, roughly, an equal-sided triangle with one side applied to the wall of the bony tube, one side to the septum that divides the bony tube into two parts, and the other stretched across the upper division of the bony tube. On the first and third of these the epithelium is of no further interest to us, and we shall study that part which rests on the septum, for it is here that the auditory apparatus is formed out of the layer of epithelial cells. The apparatus forms a band which is cut several times in its spiral course by a median section through the long axis of the cochlea.

Figure 198 represents this basal section of the epithelium of the membranous tube, as cut in the second spiral of a young Guinea pig's cochlea. The apparatus is called the *organ of Corti*.

Beginning from right to left, we find that the simple epithelium is cuboidal where it first appears on the septum. These cells have been called the *cells of Claudius*. They rise up against one of their neighbors which is grown to five or six times their height and has acquired a pointed end. This cell is known as *Hensen's cell*, and while but one of them, representing a single row, is seen in the specimen from which our drawing is taken, they form a double row of cells in the Guinea pig and several rows in extent in the cat and some other mammals.

The next six cells (representing six rows) are of two kinds placed alternately. Three of them, including the first, are tall supporting cells with narrow upper bodies that expand at the tip into plates. These plates reach from one cell to the other and to the tip of the Hensen cells

to form an almost continuous cover. These supporting cells are known as the *Deiter's cells*. This cover is interrupted at regular intervals to permit the tops of the short outer auditory cells or outer cells to form a part of the surface in the row. These short cells do not reach to the basement membrane, but are supported in their elevated position by the three rows of Deiter's cells.

The next cells appear in two rows, which are composed of the tallest cells of all. They spring from broad bases wide apart and lean toward each other at an angle with narrow bodies which touch and merge by a wide, curved contact at the top. The first of these two rows are the *outer* and the second the *inner pillar cells*. The round tube-like space

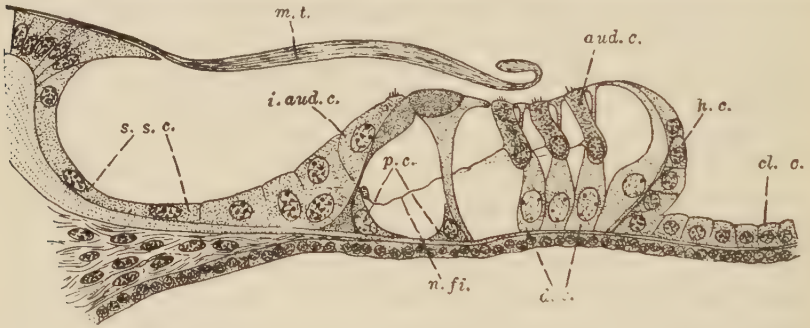

FIG. 198. — Section of the organ of Corti of a young Guinea pig, *Cavia*. *cl.c.*, cells of *Claudius*; *h.c.*, Hensen's cells; *d.c.*, Deiter's cells or supporting cells of the sensory epithelium; *aud.c.*, auditory cells or hair cells (outer); *p.c.*, outer and inner pillar cells; *i.aud.c.*, inner auditory cell or hair cell; *n.fi.*, nerve fibrils; *m.t.*, membrane tectoria; *s.s.c.*, cells lining the sulcus spiralis.

that runs between them is the *tunnel of Corti*. The greater part of the cell body is a specialized product of the cytoplasm.

Next to the inner pillar cells is found a single row of hair cells, the inner hair cells, or, as we shall call them, the *inner auditory* cells. They are followed by a portion of simple epithelium that lines a groove called the *sulcus spiralis*. This epithelium is thicker than that which is continued over the remainder of the septum and across as the free *membrane vestibularis* to the outer bony wall and thence to the point at which we began, the *cells of Claudius*.

The nerve supply consists of the different processes of neurons lying in a ganglion that is found in the immediate neighborhood. These fibers pass along through the bony septum which is called the *lamina spiralis* and send naked fibrils in a bundle to the hair cells. Some of these fibrils form perceptory end-plates on the inner hair cells and the rest cross the tunnel of Corti, and, passing into the spaces between the sustentacular cells and under the hair cells, end in the same way on the outer hair cells.

We have here ample proof that the hair cells, both outer and inner, are the *sensory auditory cells*. The stiff, short rods set in their upper surfaces are the *cell-organs* of auditory *perception*, and receive the stimulus.

Our next concern is to know as well as we can how this stimulus is imparted to the auditory rods. Do the waves of sound vibrate them, or is there an intermediate tissue or substance?

As in the tactile and static tissues, we find that here an intermediate substance is present and is probably necessary. This is a plate or shelf of material which projects from the elevation on the left, the *labium vestibularis*, and reaches across to cover the hair cells with its edge. It is called the *membrana tectoria* and is probably a cuticular product of the labial cells. Such a broad surface must easily be made to vibrate by the waves of sound, especially as they come intensified by accessory tissues, and must thus play mechanically upon the auditory rods and give them a characteristic stimulus.

The accessory tissues are the pinna, a shell-shaped organ designed to catch a volume of sound waves, and, having concentrated them, to project them through a tube and against a stretched membrane, the *tympanum*. In a frog this tympanum is larger and directly exposed to the air waves without the aid of such accessory tissues.

The tympanum is composed of both connective-tissue and epithelial elements, and the four layers are, from without inward, a thin stratified epithelium called the *stratum cutaneum;* a layer of connective-tissue fibrils arranged as a radiating tendon and known as the *stratum radiatum;* another connective-tissue layer called the *stratum circulare;* and an internal layer of simple cuboidal epithelium that is continuous with the epithelium lining the middle ear. The three tiny bones which form a chain to transmit the vibration to the internal ear-sac are composed of a very fine and dense bone tissue.

Technic. — The same remarks as to technic may be applied here as were found following the previous part. It may be added that no careful studies of a tissue of hearing can be satisfactory if they are not grounded on experimental work that demonstrates the tissues to be actually sensitive to sound stimuli. The auditory tissues of insects are very hard to handle by the section method, owing to the chitinous structures with which they are associated. When the insects are small and have a delicate cuticle, the sections may be easily secured. Also when a larger insect with a heavy cuticle has just emerged from the molt, its shell is soft and may be ignored. Chitin can be softened, but always at the expense of the other tissues. It is better to remove the chitin if possible.

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TISSUES OF LIGHT PERCEPTION

Light is produced by short, rapid undulations of the invisible ether. These movements are capable of producing chemical and physical changes in living and dead matter. Their effect on ordinary animal cells is not perceptible at the nerve centers. By some, they are thought to be a stimulant and, in too great quality or intensity, a poison to ordinary protoplasm. Thus the surface of most animal bodies is fitted to keep the light from all underlying parts (see Chapter XIII, Pigment).

Upon *certain of the body cells*, however, light does leave a definite impression. There are some nerve cells that not only perceive the light but are stimulated by it to send a report of the fact as an impulse to a nerve center, either through their own efferent process or through communicatory nerve cells that form a path. Such cells are known as the *visual cells*, *retinulæ*, *rod-cells*, etc. We shall call them the *visual cells*. The light is given access to them through accessory tissues made transparent for the purpose.

The visual cell, in its specialized state, has developed a specific cell organ, a peculiar, rod-like structure secreted or otherwise formed by the cytoplasm and capable of being stimulated by the ether waves. It is weakly developed and almost invisible in some low sight cells, while in others it is larger than the cell which produced it, and so clearly differentiated that it appears to be a separate structure. It is, where careful observations have been made, laminated, and the separate plates or rods of which it is composed usually lie at right angles to the light which stimulates it. Its exact chemical and physical relations to the light waves during stimulation are not known. It can be stimulated by other factors than the light waves, as pressure and chemical activity, but it gives, under these circumstances, the same impression to the brain

centers as if it were affected by light waves. It cannot perceive all light waves, and varies considerably in the number of kinds of them that can stimulate it. This cell-organ of sight is known by various names, of which we shall use but two, the *rhabdome* or the *visual rod*.

The rhabdome is placed in various positions on the cell body. It may be on the edge or on the end of the cell and may assume any position of its body to fit in with other optic structures. More rarely it is found inside the cell. It may even be formed upside down in case the cell receives its light rays from behind (eye of man and *Pecten*). It is not known if the rays of light, striking the plates from their rear or from any other direction than in the front, can stimulate the cell.

The visual cells are sometimes found scattered on the body surface, but are usually collected into one or two, or even many, groups which, together with the accessory tissues, are called the *eyes*. Eyes may perform three functions for their possessor: to perceive the light according to its intensity, or to perceive it according to its direction, or to record light-images of the objects from which the rays come. Some eyes do all of these things.

To perceive the light alone according to its intensity is a function of the individual visual cell, as well as of the highest eyes. To determine its direction depends upon the position of the visual cell with reference to the body or some larger part of the body. It is also determined by the position of a cell or group of cells stimulated to the exclusion of the rest of the retina. To perceive an image depends upon the relations of many neighboring visual cells to each other and the presence of properly arranged brain centers that can receive their numerous reports as a related whole.

Some forms of visual cells probably exist that have not been pointed out to science. The surface of the body, in some animals, contains other light-sensitive cells than those in the eyes, and these can perceive light and even the direction of light. The frog forms a concrete example. If deprived of its eyes, a frog will still be able to orient itself with reference to a ray of light.

The most primitive form of visual organ would consist of certain simple epithelial cells, different from their fellows only in their development of a visual cell-organ or region and their consequent ability to perceive light. An organ of this kind probably exists in such animals as the earthworm, the frog, and some medusæ. This brings us once more to realize that very simple eyes will sometimes be met with in highly organized animals. We shall learn later that some simple or lowly organized animals have quite complex eyes, and further, that both kinds of eyes may exist in the same animal.

Pigment is almost always found in connection with the visual cells.

Its function seems to be the secondary, but important, one of protecting the visual cells or their rods from undue amounts of light as well as forming an absorbent background that will free them from unnecessary reflections. This pigment is sometimes in the visual cells themselves, but more often in other cells that are specialized for this function and found in close connection with the visual cells. It appears as the usual clouds of fine, dark brown granules in the cytoplasm of the cells.

Some tissue cells in the neighborhood of the visual cells are modified so that their entire body becomes exceedingly transparent and of a high index of refraction. These cells also, either individually or collectively, assume a spherical or other curved form which serves to collect and thus concentrate the power of the rays or even to arrange them as an image on the layer of visual cells. Such a refractive body is the *lens* of the eye. The *lens* is not always a cellular structure. Many consist of transparent cell-products, as the cuticular lens of the arthropod eye or the gelatinous lens in the visual organs of some worms.

In a few rare cases the lens as an image former is done away with entirely, and a diaphragm in front of the retina is used to produce an image, on the principle of the pin-hole camera. This diaphragm also serves to determine the amount of light to be admitted to the retina, and in this capacity it becomes known as an *iris*. A well-developed iris is found in some eyes that also have a lens, and it serves here to regulate the amount of light that shall enter. It does not take part in the image formation when a lens is present.

When arranged to receive an image, the visual cells, together with some accessory cells, are known as a *retina*. Many communicatory nerve cells are included in this structure. They sometimes form part of the layer and sometimes are removed from it in ganglia where they have a peculiar arrangement in layers that evidently have some relation to the retina.

The above structures are all very delicate and, for the most part, must be protected from contact with the exterior. For this purpose we find some cells that make it their duty to form protecting coverings that shield these delicate structures. Such an organ is known as a *cornea*, and may be composed of a single layer of epithelium, or of an epithelium lying on a connective tissue or of no cells at all, as in the arthropods, where it is the chitinous secretion of certain outer cells. One prominent feature of all cells and tissues that take part in the formation of a cornea or a lens is their *transparency*. The necessity for this is apparent.

Visual organs in the Protozoa. — The Protozoa that are unquestionably animals show no clearly defined light-perception region. The general surface of the cell is sensitive to certain *intensities* of light, the dorsal surface probably being more sensitive than the ventral region.

Certain unicellular forms, mostly probably plants, have light-perceiving structures. Here a certain region becomes furnished with a disk or cup-shaped pigment spot and a cuticular lens (Fig. 199). In this region the cytoplasm is more highly sensitive to light. This is primarily a structure for perceiving the intensity of the light. But, as it is always placed eccentric to the long axis of the plant, and, since the plant moves about, rotating on its long axis, it becomes also a device for perceiving direction of light rays. In colonial forms, presenting these so-called

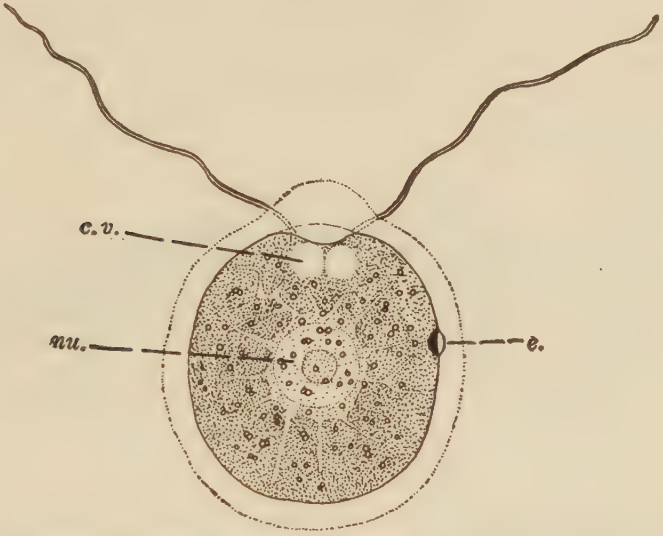

FIG. 199. — Individual of the flagellate, *Chlamydomonas reticulata*; *e.*, eye-spot with pigment and lens; *nu.*, nucleus; *c.v.*, contractile vacuole. × 1000.

stigmata, the stigmata are always arranged with reference to aiding the colony to determine the direction of the light source.

We can find no homogeneous basis upon which to classify the visual organs of the Metazoa, owing to the way in which the prominent features of these organs are distributed among the various examples. There seems to be very little homology among them based upon a common ancestry, and the same animal will often have two different kinds of eyes on different parts of its body, or even near one another on the same part. We shall therefore consider them in groups that are rough associations of eyes of somewhat the same degree of tissue complexity, or else which belong to closely related groups of animals. Comparisons of these, from the tissue standpoint, form an interesting study. We shall begin our study of the eye of Metazoa with a very simple eye.

The eye spot of a plecypod mollusk, *Solen*, as described by Sharp, is probably the simplest true visual organ that has a demonstrable struc-

ture (Fig. 200). The epithelium on the mantle edge showed pigmented areas. As pigment is so often associated with light perception, experiments were tried, and it was found that the mollusk reacted to light on these spots and on them only.

Sections reveal a thickening of the epithelium due to the simple lengthening of the columnar cells. Their distal ends have acquired a rather heavy mass of black or dark brown pigment. Otherwise, no specific rhabdome is to be seen.

A feature of this spot which helps to decide that it is an eye is the transparent thickening of the very slight cuticle which is formed in this region, into a flat lens. This lens is arranged so as to rather weakly concentrate the light rays on the most pigmented cells. A form without this cuticular lens would represent the very simplest eye.

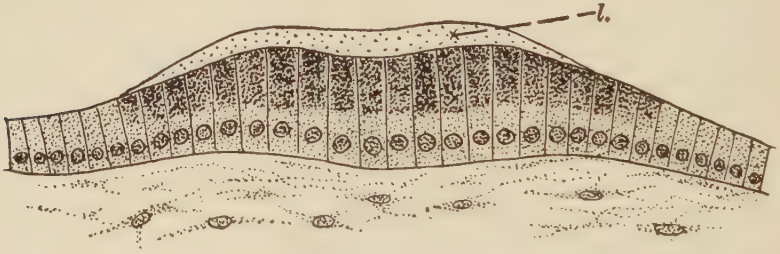

FIG. 200. — Vertical section of an eye-spot on the mantle edge of *Solen vagina*; *l.*, lens. (After BENJAMIN SHARP.)

The eyes of some starfishes furnish still other examples of a very primitive light-perceiving organ. These forms also show, among their different species, a gradual succession of stages which may be considered to indirectly represent phylogenetic steps in the development of the echinoderm eye. A very simple form is to be seen in *Astropecten Mülleri* (Fig. 201). In this animal the simple epithelium of the body is modified, over a portion of the radial nerve called the eye cushion, so that it consists of two kinds of cells.

The first are the supporting cells (*sup. c.*, Fig. 201) which, besides acting as support for the surrounding tissues, are used to produce from their distal ends the thin, double-layered, outer cuticle (*cu.*). Proximally they rest on a basement membrane (*b. m.*) which separates them from the underlying connective tissue and muscle.

Placed between these supporting cells, either singly (rare) or in groups of from two to five or more, as seen in the section, are the other modified epithelial cells, the light-perceiving cells or visual cells (*vis. c.*). They are stouter but more delicate in texture, and the nucleus is placed as a rule farther distally in the cell than was the case in the supporting cell. The distal end does not rest against the cuticle, but projects as a

rounded end into the space between the cuticle and a parallel inner membrane called the *limiting membrane*. This space is filled with a fluid in life, and the ends of the sensory cells which pass into it through the limiting membrane are the cell-organs of light perception or *visual rods*.

The proximal ends of the visual cells do not reach to the basement membrane, but are prolonged into delicate nerve fibers to conduct the impulses to the nerve centers. These nerve fibers are seen in the drawings as sections of numerous fibers which lie among the base of the tall and thin supporting cells.

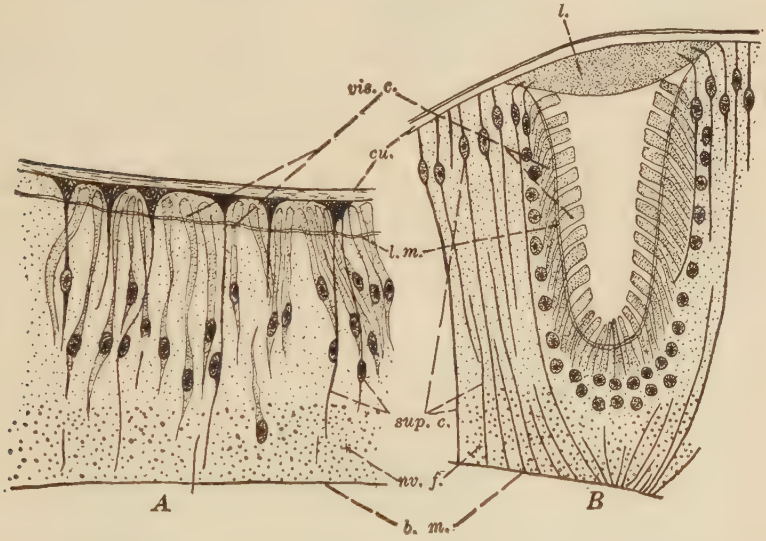

FIG. 201. — Parts of retinal tissue from the eyes of (*A*), *Astropecten Mülleri* and (*B*), *Asterias tennispina*; *vis.c.*, visual cells; *sup.c.*, supporting cells; *l.*, lens; *cu.*, cuticle; *b.m.*, basement membrane; *l.m.*, limiting membrane; *nv. f.*, nerve fiber layer. (After PFEFFER.) × about 600.

This eye has no lens and is of extreme simplicity. Drawn in the same figure with it is a representation of an eye from another starfish, *Asterias tennispina*. On the optic cushion of this echinoderm the visual cells are not distributed diffusely as in the preceding example, but are collected into groups to form more definite "eyes." Each of these groups is depressed into a cup-like hollow, carrying the limiting membrane with it and displacing to some degree the surrounding supporting cells. Such of these latter as immediately surround the depression bend over and touch the inner surface of the cuticle, and, besides forming and supporting this cuticle, they deposit on its inner surface a delicate, flat lens (*l.*). The lumen of this depression or invagination remains open and is filled, in life, with a fluid. The sensory cells form

nerve fibers from their proximal ends, and these fibers unite to form the same nerve tract that was to be seen in *Astropecten*.

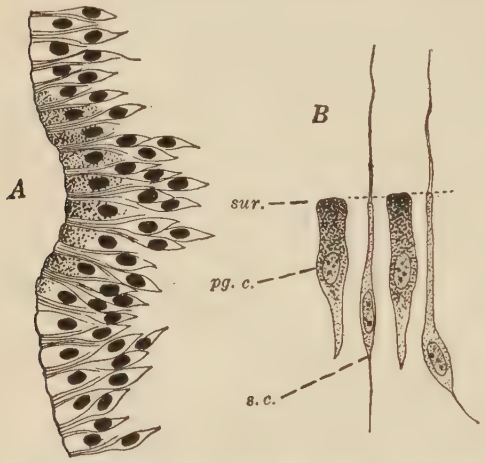

FIG. 202. — Eye of *Charybdea marsupialis*. *A*, general view; *B*, greater magnification of four cells to show the alternation of sensory cells (*s.c.*) with pigment cells (*pg.c.*); *sur.*, outer surface; A × 760. (After SCHEWIAKOFF.)

It should be noticed that these eyes are slight advances on the eye of *Solen*, because of the development of a visible rhabdome or visual rod on the sensory cell. In both of the above visual organs the light strikes the perceptory cell *directly*, and from a distal position.

One more of the extremely simple eyes should be studied in a medusa, *Charybdea marsupialis*. This animal has two very different kinds of eyes on one and the same part of its body. The simplest is shown, in a vertical section, by Figure 202. Here we again find the pigment cells having very much the same appearance as they had in *Solen*. But the point to be noticed is that these pigment cells are not the sensory cells, this function having been left to alternate visual cells which have developed a sight rod for the purpose. These visual cells are differentiated out of the same primitive epithelium from which the pigment cells were derived, and two of them are pictured in the figure, much enlarged and almost in their natural relations to the pigment cells, a slight space being left for the sake of clearness. The complex eye of

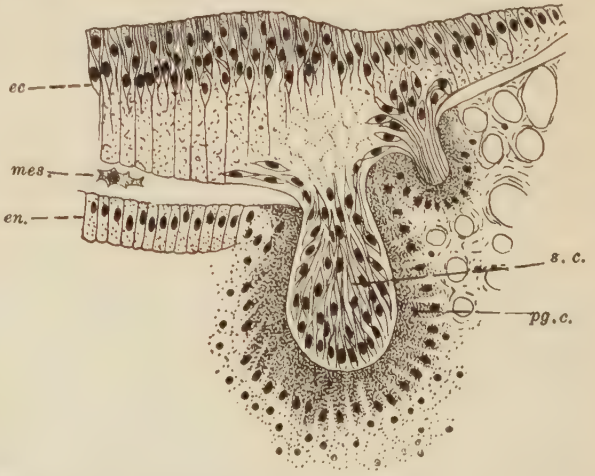

FIG. 203. — Section of the double eye of *Aurelia aurita*. *s.c.*, visual cells; *pg.c.*, pigmented cells; *ec.*, ectoderm; *en.*, endoderm; *mes.*, mesogloea; × 760. (After SCHEWIAKOFF).

Charybdea we shall not describe, its type being represented later by other forms.

Another medusa, however, has an intermediate type which should be considered here. This form, *Aurelia aurita*, has one part of its body-wall, consisting of all three layers (the mesoglœa weakly represented), developed into an eye that is a simple *inverted* type. That is to say, the light passes through the cells and strikes their visual cell-organ from a proximal position. This eye is pictured in Figure 203, and we see that a portion of the animal's ectoderm has been proximally produced in two places to form two knobs, one of which is larger than the other. These knobs are composed of long sensory cells of ectodermal origin, which rest on the inner surface of a pocket-like layer of cells which have been invaginated distally (or evaginated if they are considered with reference to their distal surface) to form these pockets.

The cells which line the pockets have developed pigment in their proximal ends (these ends are directed distally with reference to the animal's exterior), and form the protective and absorptive layer of the eye. There is no lens connected with this eye, although some medusæ have a well-developed one. The mesoglœa seems to be crowded out entirely in the structure, and the figure shows but one cell belonging to this layer and lying at some distance from the point at which the eyes are formed.

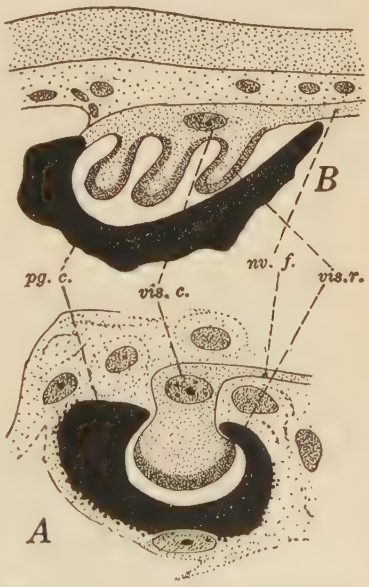

FIG. 204. — Axial section of a single eye of *Planaria torva*. *B*, a similar section of a side eye of *Amandia polyopthalmia*; *vis.c.*, visual cell *nucleus*; *pg.c.*, pigment cell; *vis.r.*, sensory cell-organ of light perception, corresponding to visual rod; *nv. f.*, nerve fiber of visual cell extending to central ganglion. (After R. HESSE in *Zeits. f. wiss. Zool.*)

The eye of a planarian worm furnishes an example of a simple eye of slightly greater complexity than of the starfish and *Solen* and with more highly specialized visual cells than in the medusa eyes which have just been described. We shall study an eye from *Planaria torva* and another from *Amandia polyopthalmia* (Fig. 204), and at the same time compare them with the larger eye found in another planarian worm, *Planaria gonocephala*.

Among the many eyes distributed over the dorsal surface of *Planaria torva* are some composed of a single visual cell. As in most planarians, this is sunk beneath the

epithelial surface from which it originated and lies within the body tissues, one of whose cells has moved alongside of it and developed black pigment in some of its cytoplasm. The pigmented portion of the body is arranged in a crescentic form, with the nucleus placed on the outer side in a small portion of undifferentiated cytoplasm.

The distal cytoplasm of the visual cell is much enlarged, and forms a mass that is bent laterally and lies in the concave side of the pigment cell. The distal edge of this mass is modified into a denser substance, the cell-organ of sight or rhabdome, which forms a lining against the interior of the cup-shaped pigment cell.

It should be noticed here that this visual cell is inverted; that the rays of light must pass through the cell body and nucleus as they enter the opening of the pigment cup and strike the rhabdome from its proximal side. The efferent pole of the visual nerve cell is drawn out into a fiber that passes inward, to some ganglion where its impulse can be discharged and used.

Concerning the central connections of this fiber but little is known. It can be said, however, that it forms a very simple pathway for the light-perception impulse, the greater part of the distance being furnished by the one drawn-out process of the visual cell itself. This is not true in any eye that forms an image.

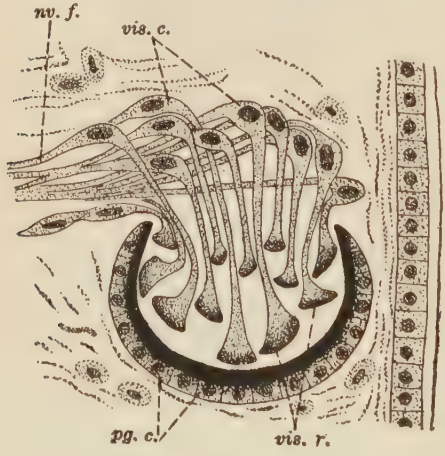

FIG. 205. — Axial section of the eye of a planarian worm, *Planaria gonocephala*. *vis.c.*, visual cells; *vis.r.*, visual rods or rhabdomes; *nv.f.*, centripetal fibers of visual cells; *pg.c.*, pigment cells. (After R. HESSE in *Zeits. f. wiss. Zool.*)

The eyes of other planarians are often but larger forms of this monocellular organ with its monocellular pigment cup. In the eye of *Amandia* (Fig. 204, *B*) there are three or five visual processes from the one cell, with the perceptory organs on the ends of the processes. These processes are directed into the same kind of a mononuclear pigment cup.

In *Planaria gonocephala* we find the same kind of an organ, except that there are from twenty to thirty or more visual cells, and the pigment cup is made of a layer of over a hundred separate cells instead of only one cell. As in the unicellular form of pigment cup, the nucleus is always in the proximal part of the cell body and the pigment is in the distal part (Fig. 205). A slight difference is to be seen also in the

visual cells. The perceptory organ is separated from the cell body by a cytoplasmic process instead of resting directly upon it. The afferent process is thus almost a fiber, like the efferent process.

All these planarian eyes have sunk below the surface, and the break in the outer epithelium has been grown over by its simple cubical cells, which thus form a *cornea*, together with the connective-tissue elements between them and the eye. But it is an unspecialized cornea and not different in any way from the rest of the integument. There is no lens present.

Another eye type of which space forbids a full description is that seen in the leech. Here also we meet with an organ that contains from one to upward of forty visual cells. The degree of specialization is a high unicellular one. The visual cell-organ is peculiar, and contains in its body both retinal and lens structures.

Going back to the echinoderms, in which we found the simplest type of eye in a starfish, we find our next step of development materialized in one of the urchins, *Diadema*, where the eyes are developed on the bases of the spines.

This eye is somewhat compound; that is, it is composed of a number of units, any one of which would represent an efficient organ of light perception. These units are each composed of several different cell groups or tissues, one of which, the superficial cornea, is common to all of them. Each unit is an upright, five- or six-sided column whose proximal end is somewhat pointed and rests in a pigment cup. This pigment cup is composed of mesodermal cells which have moved up from below and formed the pit-like cavity which opens distally. The cup forms the sides of the eye-unit or *ocellus*.

The larger body of each ocellus is an oval mass, made up of ten or twelve large transparent and refractive cells with small nuclei. These cells are elongate and roughly wedge-shaped with the sharp ends pointed toward each other and interlocking, while the blunt ends rest on the surface of the mass. This organ is undoubtedly the lens, as can be told by its transparent and refractive nature and the position which it occupies.

The lens is capped distally and proximally by two caps, each composed of a single layer of cuboidal cells and each covering nearly a third of the lens surface. These caps are well shown in two middle ocelli, where they are represented as in a surface view (Fig. 206). In the ocelli, to right and left, they are seen in median section only. They are much alike, and only the position seems to decide that the proximal cap must be composed of visual or light-perceiving cells. The upper cap has been spoken of by the Saracens as a germinative group of cells from which the lens is formed and removed.

As has been indicated, a common covering of a simple ciliated epithelium covers all the ocelli. It is roughly divided into fields by the slight curvature of the outer ends of the ocelli.

Below, the ocellus is set into the pigment cup with its lower or visual cap of cells lying in direct contact with the pigment cells. Nerve fibers come up from the sub-dermal nerve layer and provide the ocelli with fibrils. The eyes are evidently very efficiently coördinated with the nerve centers, because, if the light is interrupted from any quarter, the animal at once moves all its wicked spines so that they point to this quarter and are ready to repel an attack.

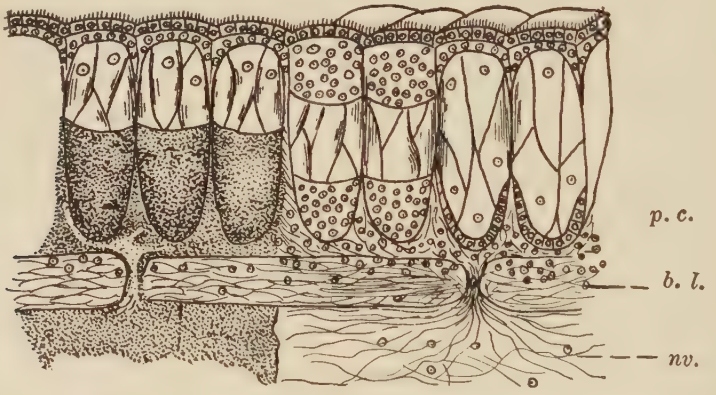

FIG. 206. — Vertical section through an eye-spot of *Diadema*. Slightly schematic. *nv.*, nerve layer which sends branches to eye through the basal connective-tissue layer (*bl.*); *p.c.*, proximal layer of sensory cells. The three eye units to the left show pigment caps on bases. Those on the right are complete median section. The two in the middle are lateral surface views to show the outer and inner cell caps. (After C. F. and P. B. SARASIN.)

Although they are far more specialized in most ways, **the Arthropods show a type of eye** that should be studied at this point on account of structural characters which lead one naturally to think of them when studying the eye of *Diadema*. This eye as well as *Diadema's* is called compound because it is composed of a number of similar units, each of which is apparently independent of the others so far as seeing is concerned. When the nerve centers with which this eye is connected are studied, however, it is apparent that the eye must act as a whole in some manner.

A characteristic feature of this eye is the way in which the cuticle is carried into its formation. The cuticle seems to be a more predominant feature in this group than in any other.

We shall study the eye of *Periplaneta orientalis* as **a type of the arthropod eye**, referring to a crustacean form, *Palæmon squilla*, for occasional comparison. The outer surface of these eyes shows a rather

weak division into very many (perhaps 10,000) divisions, and a vertical section through the principal axis of the eye (Fig. 207) will show that each of the superficial divisions represents the base of a long, thin truncated cone, whose inner and smaller end rests on the small semicircular basement membrane, on which the ends of all the other cones also rest. Each of these cones represents a single unit or *ommatidium* of the eye, and it is made up of the following kinds of cells and cell-products.

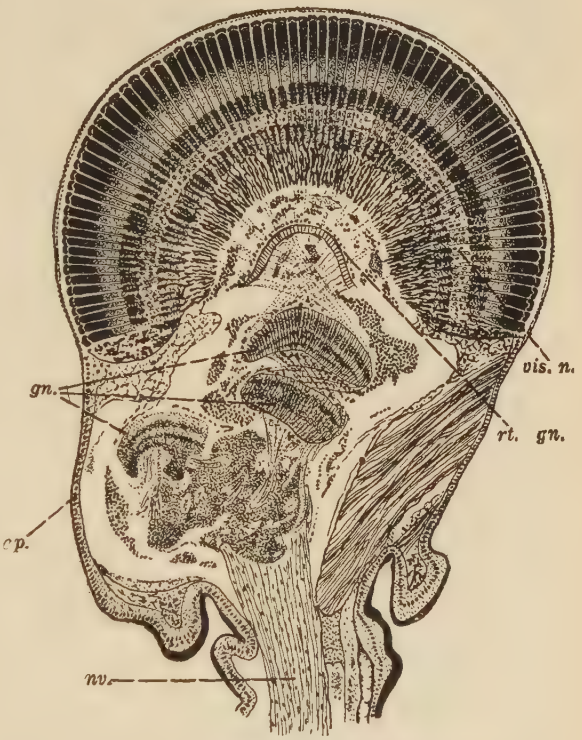

FIG. 207. — Axial section of the eye and eye-stalk of *Palæmon squilla*. *gn.*, optic ganglia; *ep.*, epidermis (hypodermis); *vis.n.*, visual cell nuclei; *rt.gn.*, retinal ganglion; *nv.*, optic nerve. (After SCHNEIDER.)

Rising up from the basement membrane are a circular group of *retinula cells*, which are *visual nerve cells*. In both *Periplaneta* and *Palæmon* these are seven in number. In *Palæmon* (Fig. 208) they rise together equally, with their distal ends enlarged and containing each a nucleus. These nuclei are thus at nearly the same level. In *Periplaneta* (Fig. 209) three of the cells have the larger mass of cytoplasm, which is lower in the general cylinder mass, and the remaining four nuclei are thus at the top. Cell boundaries are hard to see between these cells, and so a longitudinal section of an ommatidium of *Periplaneta* appears to have two retinula cells cut in longitudinal section and possessing each an upper and a lower nucleus.

The inner edge of each visual cell has, developed from its cytoplasm, a cell-organ which has been called a *rhabdomere*. As all the rhabdomeres fit closely together, they form a single spindle-shaped or club-shaped organ called the *rhabdome* (we shall hereafter speak of the single

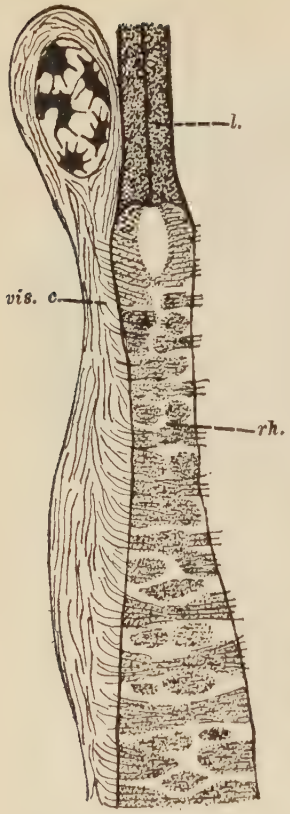

FIG. 208.—Part of an ommatidium from the crustacean, *Palæmon squilla*. *rh.*, rhabdomes; *l.*, lens or crystalline cone; *vis.c.*, visual cell showing a nucleus near its top. (After SCHNEIDER.)

cell-organ, mentioned above as a rhabdomere, as a *rhabdome*). This is the only part of the whole eye that the light-waves can affect so as to produce a stimulation, and the rhabdomes transmit this stimulation to the retinula cells as an impulse, which is carried out to the optic nerve centers through the proximally produced cell bodies of the retinulæ, or visual cells, which, therefore, serve as nerve fibers. Fine nerve *fibrils* have been detected in the rhabdomes of *Palæmon*, and they pass through into the retinula cell (see Fig. 208). In fact, it can be seen in *Periplaneta* that the whole rhabdome is nothing but an edge of the retinula cell bearing a row of innumerable tiny parallel bristles or rods. These rods come out at right angles to the axis of the cell, and are therefore at right angles to the light-waves. They form the plates spoken of in the first part of this chapter.

Over the expanded ends of the rhabdomes lies the end of the lens. This is composed of several parts, four in number in most arthropods, and these parts are formed by four thin cells which lie just outside of and around them. These are the *lens-cells* (see Fig. 209). They rest proximally upon the retinula cells according to Grenacher; while according to Patten they pass down to the basement mem-

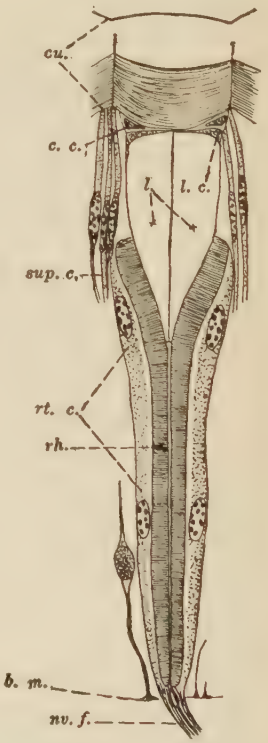

FIG. 209.—Longitudinal section of a single ommatidium of *Periplaneta orientalis*. *b.m.*, basement membrane; *cu.*, cuticle divided into corneal areas; *rt.c.*, retinula cells or visual cells; *rh.*, rhabdome or cell-organ of light perception; *l.*, lens; *l.c.*, lens-cells; *c.c.*, corneal cells; *sup.c.*, supporting cells (slightly modified hypodermal cells); *nv.f.*, nerve fiber. (After R. HESSE in *Zeits. f. Wiss. Zool.*)

brane outside of them. Grenacher's view certainly accords with the facts as seen; while Patten's view seems to represent the condition which should obtain if both kinds of cells were derived from the same layer by a simple process of differentiation. It is probable, however, that the retinula and lens-cells were derived from the upper epithelium by delamination, through successive amitotic divisions, as a terminal process. This would support Grenacher's view.

Lying still distad from the crystalline cone and usually separated from it by the cone cells are four *corneal cells*, which are the hypodermal cells that produce the cornea. They are flat and thin, and in the insects two of them become pigment cells (see Fig. 209, *c. c.*). Other pigment cells are found around the ommatidium. They are mesodermal in origin, and some of them expand distally to cut off an excess of light, thus acting as iris cells.

There is an almost infinite variety of arthropod eyes, differing principally in detail from the two examples. This detail is so extensive, however, that we cannot begin to discuss it. We shall examine two other arthropod eyes, the **accessory eye or ocellus of an arthropod** and the eyes of an arachnid, to see a simpler type of visual organ, but one which is yet capable of a higher differentiation than the extremely specialized insect and crustacean compound eye. The ocellus of a *Dytiscus* larva shows a simple invagination of an area of the hypodermal cells (Fig. 210). Those which have left the surface have lost all cuticle-forming power, while those which are still at the surface and near the closed point of invagination have secreted an extra amount of cuticle as a lens.

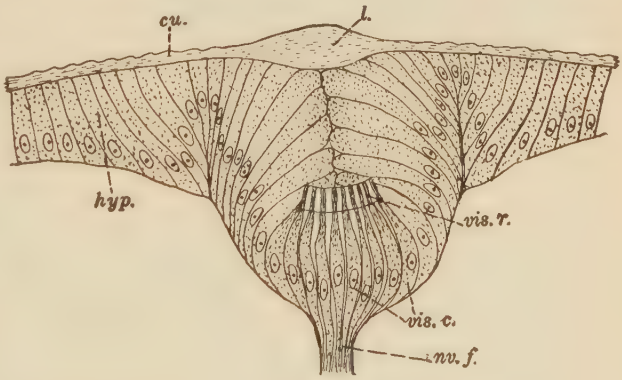

FIG. 210. — Axial section through ocellus accessory eye of the larva of a beetle, *Dytiscus*. *cu.*, cuticle; *l.*, lens; *hyp.*, hypodermis; *vis.c.*, visual cells; *vis.r.*, sensory or visual rods on visual cells; *nv.f.*, nerve fibers derived from bases of visual cells. (From LANG after GRENACHER.)

The cells lying in the fundus have developed visual organs in the form of small rods which point distally. The proximal ends of these cells are developed as usual into efferent nerve fibers (see Fig. 210).

These cells, which line the sides of the invagination, meet across the

line of vision, but their distal ends are rendered transparent so that the light can pass through. They occupy a position in which cells similarly placed in other eyes would form a lens. It is even possible that, owing to different indices of refraction in different regions of the cell body, some sort of lens function is performed, but the presence of the corneal lens above tends to invalidate such a mere supposition. They may be called the glassy or vitreous cells.

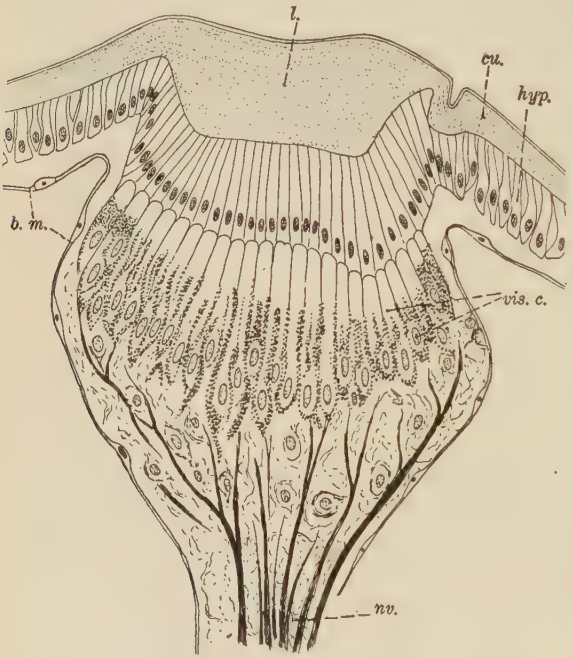

FIG. 211. — Axial section of an ocellus of the orthopterous insect *Perla bicaudata*. *cu.*, cuticle which is thickened at (*l.*) to form the lens; *hyp.*, hypodermis which is thickened centrally to secrete the lens and to constitute the crystalline body; *vis.c.*, visual cells which were derived from the modified central hypodermis or crystalline layer; *b.m.*, basement layer of connective tissue; *nv.*, nerve composed of processes from the visual cells. (After REDIKORZEW in *Arch. f. mik. Anat.*)

In the majority of other insects the retina layer of the ocellus is derived from the hypodermis in a different manner. The first step consists of the invagination of the hypodermis, on the area that is to be occupied by the ocellus, into a shallow pit. The hypodermis so depressed then separates into two layers, by a proximo-distal division of the cells according to some writers, more probably by the recession of some of the cells of the single layer into a more proximal position, where they become converted into visual cells by the development of rhabdomes and the extension of the proximal portion of their bodies into efferent nerve fibers.

The original layer of hypodermal cells, after the retinal cells have been withdrawn, becomes specialized for the transmission of light and also secretes the thickened area of the cuticle, which serves more or less imperfectly as a lens. Such a layer of transparent cells may be designated as the *vitreous layer*. It also functions as the *corneal layer*, since the lens which it forms is also the protective cornea. These conditions

are well demonstrated by the ocellus of the orthopterous insect, *Perla bicaudata*, which is represented in axial section by Figure 211.

The eye of Limulus is a simple form which shows an approach to the crustacean type. It is formed, much as was the ocellus of *Dytiscus*, from a surface area of some extent whose epithelium has been invaginated at numerous, regularly placed points into cup-like depressions. The lower surface of the cuticle, which is formed in *Limulus* as in other Arthropoda, all over the surface of the body, fits into these depressions, while the upper surface is even and continuous and shows scarcely any effects of the invaginations beneath. It thus forms a lens.

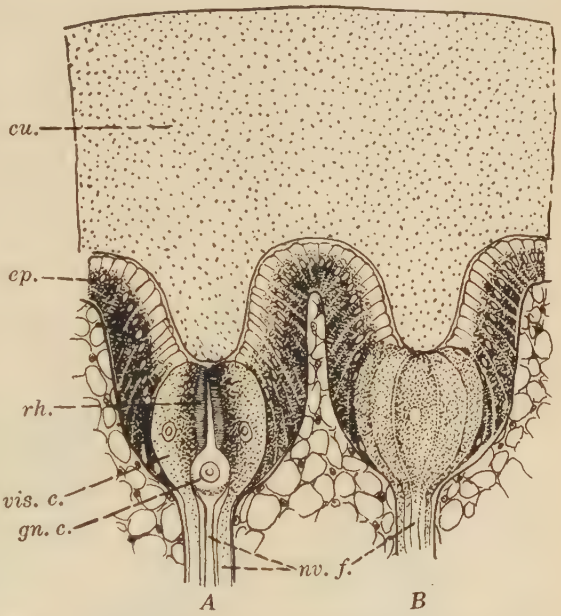

FIG. 212. — Part of an axial section of the eye of *Limulus polyphemus*. Two of the numerous ocelli are shown, one in median axial section (*A*); the other by a lateral surface view (*B*). *cu.*, cuticle, which forms a partial lens over each ocellus; *ep.*, epidermis which secretes the cuticle and from some of whose cells the visual cells (*vis.c.*) and the ganglion cells (*gn.c.*) were formed; *rh.*, rhabdome; *nv.f.*, nerve fiber. (After WATASE.)

In the bottom of each epithelial cup a group of some fifteen to twenty cells of the layer are considerably enlarged and grouped together to form a melon-shaped body, which is the retinal portion of the organ. The cells are now differentiated into visual nerve cells or retinulæ by the development of a lateral rhabdome edge and production of the proximal cell body into an efferent nerve fiber (Fig. 212).

It is in the spider that one of the most interesting visual conditions is found. This animal possesses many eyes on its dorsal surface, and both direct and inverted eyes can be found among them. Figure 213 shows a picture of a section taken vertically through two of the eyes of *Epeira diadema*. To the left is a direct eye called the *principal eye*, while to the right is the *accessory eye*, which is *inverted*. Each of these eyes was derived from an epidermal invagination which, later, was differentiated from the other. Both of these eyes consist of a simple

invagination of the surface epithelium which was then cut off and the edges of the outer layer made continuous again. This layer appears in the figure (213, *A*) as the corneal layer, and is responsible for the formation of the cuticle, which is enlarged at the point above each eye into the cuticular lens.

The method of development of visual cells on the walls of the invagination is puzzling, and the accounts of various investigators are not entirely satisfactory. The explanation in the case of the accessory eye with its inverted visual cells is evidently correct. The epithelium on the upper or distal wall of the invagination becomes the visual cell-layer. Thus the distal end of the visual cell forms the rhabdome, and the proxi-

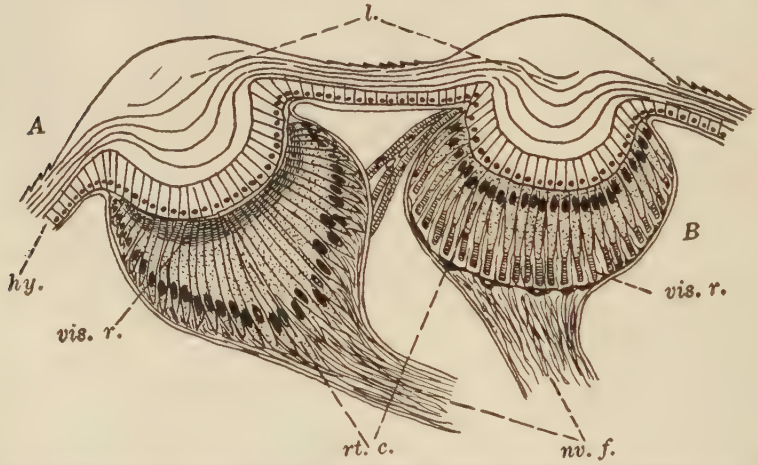

FIG. 213. Anterior and posterior eyes of the spider, *Epeira diadema*. *l.*, cuticular lens; *hy.*, hypodermal layer; *rt.c.*, retinula cells; *vis.r.*, visual rods or rhabdomes; *nv.f.*, nerve fibers. (After GRENACHER.)

mal end (now directed outward) is elongated to constitute the efferent nerve fiber. The other, or inner, wall of the invagination becomes thin and is transformed into a tapetum.

In the principal eye we have a startling exception to the usual rule that the rhabdome is distal and the nerve fiber proximal in an epithelium specialized visually. Also we have a completely lost layer to account for if the eye were formed by invagination instead of by delamination. This eye, like the other, is described as arising by a posteriorly directed, flat invagination. Its visual epithelium is also developed on the outer wall of this cavity. But the rhabdome appears in the proximal part of the cell, and the nerve processes not only are twisted around so as to leave the distal end of the cell, but they thereby are obliged to penetrate the lumen (which is here closed to a plane) and to pass through the opposite wall, which is finally lost without leaving a trace in the adult structure.

The eyes of all higher creatures are organized to form a single image, and it can be demonstrated that an image is formed by most of them. An owl's or other bird's or mammal's eye, when the posterior side is cleaned of fat, muscles, etc., and the corneal surface is pointed at a bright window, shows a beautifully distinct picture of the window on its retina from the rear. We also know that this image is transmitted to the brain and consciousness in the case of our own eye. The question arises as to how much of an image is formed by the compound eye. It has been concluded that no recognizable image is produced in some cases; while to see a male *Papillio* dart at a colored paper representation of its mate, convinces one that this insect, at least, can see and recognize form. With many other insects the eye probably does not record a form or the brain does not remember it, but it is very quick to perceive any relative motion in the objects around it.

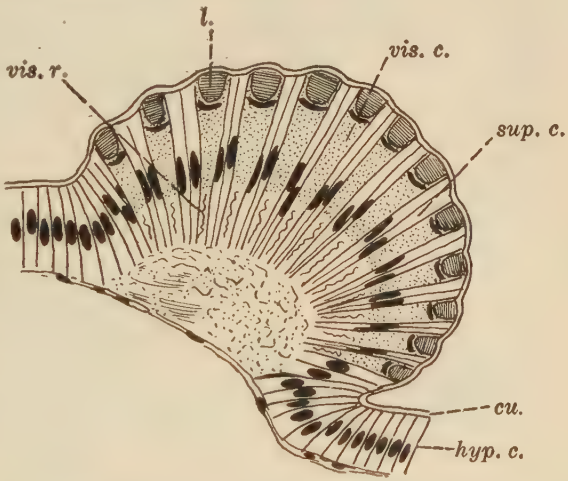

FIG. 214. — Eye from the serpulid worm, *Branchiomma*. *cu.*, cuticle; *hyp.c.*, hypodermal cells; *sup.c.*, supporting cells (slightly modified hypodermal cells); *vis.c.*, visual cells (highly modified hypodermal cells); *vis.r.*, visual rods appearing as curved hairs; *l.*, lens formed by visual cells. (After HALLER.)

One more compound eye with separate lenses may be studied in a serpulid worm, *Branchiomma*. Figure 214 represents this eye a trifle diagrammatically. The visual cells have developed out of every third epithelial cell, in section, and the intervening cells are pigment (iris) cells. The visual cell of this eye is unusual in that it secretes a lens in its distal cytoplasm as well as forming a nerve and visual cell-organ. Besides doing all this it forms or takes part in forming the cuticle. These various functions are the separate duties of differentiated cells in higher forms, as the arthropods.

The more complete yet simpler eyes, with single lens and a retina which receives an image, are found among other worms and mollusks. One has already been alluded to, but not described, in a medusa.

No worm eye is to be found that is as low in its organization as that of many mollusk forms such as *Solen*, etc. On the other hand, the mollusks have the greatest variety, some of which are among the highest

and most efficient eyes known, and far better organized than any worm's.

The forms of *Nereis* present a visual organ which well represents the average type of worm eye. Figure 215 represents the eye of a small pelagic *Nereis* of unknown species. This eye is plainly an invagination of the epidermal layer, and has been subsequently constricted off from the hypodermis at the point of invagination. The epidermal layer, after the constriction closes over, becomes the cornea with its cuticle.

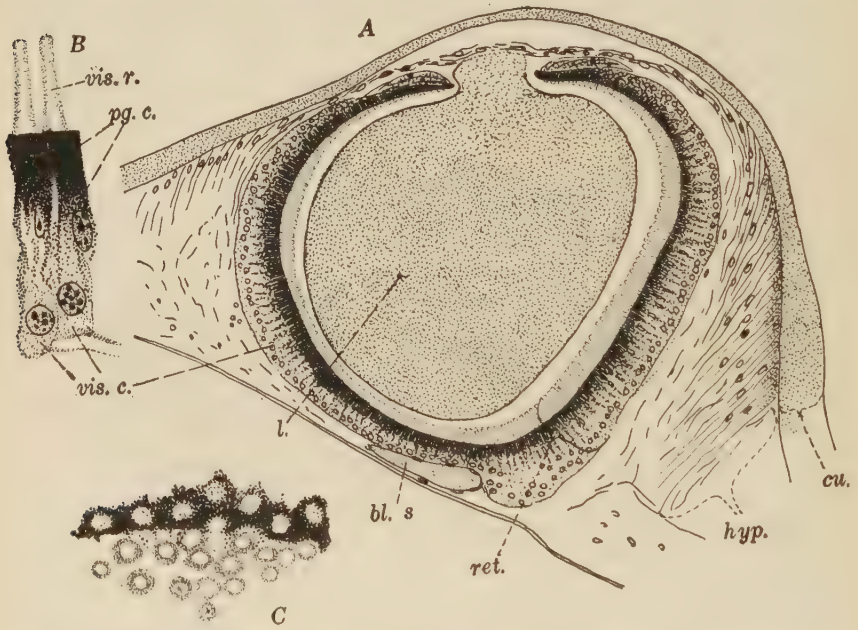

FIG. 215. — *A*, eye of a pelagic *Nereis*. *cu.*, cuticle; *hyp.*, hypodermis (a simple epithelium with very long cells); *ret.*, retina, developed by the specialization of an invaginated area of hypodermis; *l.*, lens; *bl.s.*, blood sinus. × 200. *B*, small portion of retina much enlarged. *vis.c.*, visual cells; *pg.c.*, pigment cells which surround the lower two thirds of the visual cells; *vis.r.*, visual rods. × 870. *C*, slightly oblique horizontal section across the retina near level of tops of pigment cells. × 870.

The sac-like eye changes its alternate, epithelial lining cells into visual cells and pigment cells. This takes place in a greater degree on the posterior, inside surface of the sac, and the visual specialization becomes less and less toward the anterior side, where for a short space the cells are clear and transparent to permit of the entrance of light-rays. The pigment cells of this eye form a sheet when seen from the surface (Fig. 215, *C*), and spaces in this wall denote the presence of the visual cells with their rhabdomes or visual cell-organs. This rhabdome consists of a heavy cylindrical bar of some length, in the center of which is an axial filament.

The lens, as in all worms, is an extra-cellular material secreted largely by the upper epithelium of the sac. In many other worms the lens is secreted by a single special cell which lies just outside the retina and passes the secretion into the optic vesicle through a cleft in the sensory epithelium. This occurs on the extreme inner wall, in the worm *Phyllodoce ganimosa*, the invagination of whose eye is not entirely completed. It also is seen, in a somewhat peculiar form, in the eye of *Vanadis formosa* as described by Hesse. We shall outline the structure of this eye as **a most highly specialized type of worm eye** (Fig. 216).

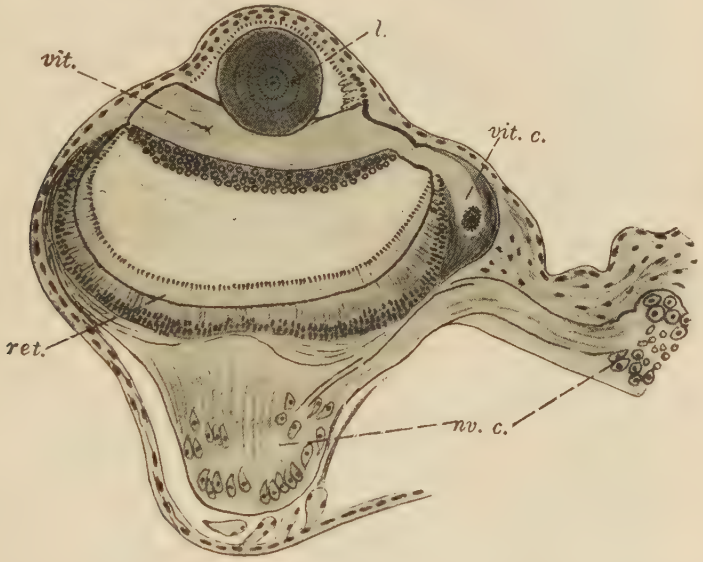

FIG. 216. — Eye of the worm, *Vanadis formosa*. *l.*, lens; *vit.*, vitreous body; *vit.c.*, large vitreous cell which forms the vitreous body; *ret.*, retina with narrow layer of pigment cells; *nv.c.*, nerve cells forming ganglia. (From HALLER after HESSE.)

The original invagination was the same as in *Nereis*, and the subsequent formation of a cornea by the surface was also the same, excepting that a small amount of connective tissue crept in between the cornea and the optic sac, as we shall call the invagination. The difference comes in the larger number and greater complexity of the structures formed by the sac, and, what is still more significant, the addition of nervous elements to the rear of the retina, probably to correlate the image elements and prepare them for their reception in the central ganglia.

The retina consists, as in the other worms, of the specialized cells on the proximal side of the optic sac. Unlike *Nereis*, however, this specialization is confined to a sharply marked area, and the visual cells are far more numerous and larger. The pigment cells are reduced to a narrow

band lying at the level of the visual cells, at the other point where these give off the visual rods.

The epithelium lining the anterior part of the sac is differentiated into two regions. That on the extreme front has secreted the round lens, a cell-product. A narrow band of the same cells, lying between the retina and the lens-forming area, produce the *vitreous body*, which probably further arranges the light-rays for their reception by the retina. One of

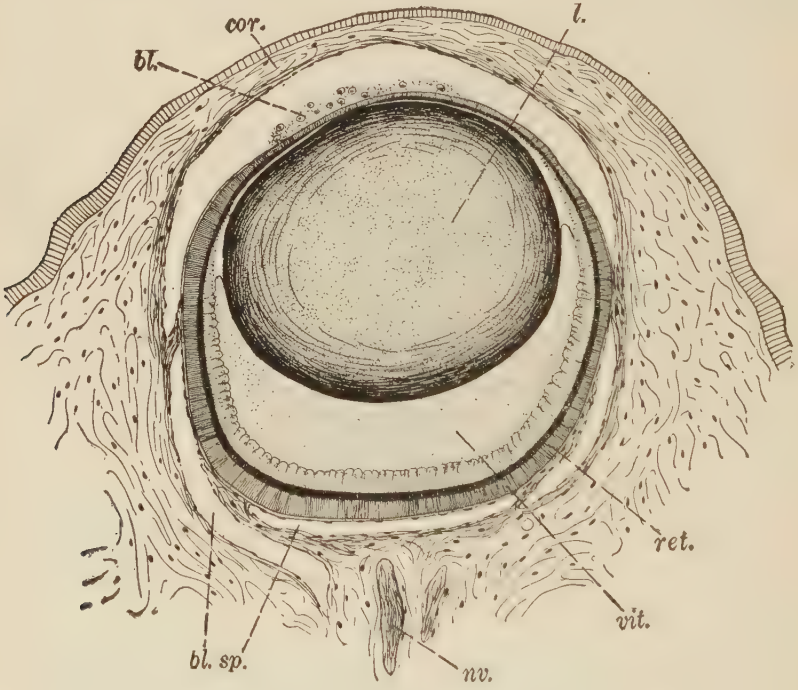

FIG. 217. — Eye of *Strombus gigas*. *l.*, lens; *ret.*, retina; *vit.*, vitreous body (fluid); *bl.sp.*, blood spaces; *bl.*, blood; *nv.*, nerve; *cor.*, cornea. × 475.

these vitreous body gland cells is far larger than the others, and has retired outside the body of the eye-sac and lies in a lateral position.

The lowest mollusk eyes have already been described. There are an infinite number which are graded from the form possessed by *Solen* up to forms that compare in their degree of specialization with the eye of *Nereis* among the worms. In *Patella rota* we find a pit such as the eye of *Solen* would form if half invaginated. *Arochus magnus* shows an eye which would represent that of *Patella* were the invagination to be almost completed and the lens material formed into a round, well-shaped ball. In *Turbo cæniformis* the structure is a complete invagination, and a cornea is for the first time formed by the cutting off of the invaginated

sac and the closing of the epidermal layers. This condition can be seen in Figure 217, **representing the eye of the common Florida conch,** *Strombus gigas.*

This beautiful eye is well developed in all particulars, although not so complex as that of the cephalopods. The eye-sac invagination has been cut off from a well-developed epithelial cornea to which a thick layer of connective tissue has been added. The sac lies, almost free, in a large blood space in which blood coagulum and blood cells can be seen. The sac is connected with the surrounding tissues by a few plate-like strands of connective tissues. The blood space is supplied with the blood by a vessel which can be traced, together with the nerve, through the entire length of the long eye-stalk.

The lining epithelium of the eye-sac is developed into the visual cells, the supporting elements (and several other cells). This is done in a far greater degree on the posterior side of the sac, where these cells form the retina. This retina diminishes in thickness as it is examined more toward the front, until first the rods suddenly terminate and later the pigment is lost, likewise abruptly, and then there is left only a very thin layer of simple epithelium lying on its outer basal membrane and forming a transparent layer to let the light-rays pass in.

This anterior region of the eye-sac is in direct contact with the lens, a spherical body with a denser outer shell. The lens is a non-cellular structure probably formed by the anterior cells of the sac which touch it. It is not as large as the inside of the cavity of the sac, but there remains between it and the retina a crescentic (in section) space filled with the vitreous fluid. In two other worms, it will be remembered, this fluid was secreted by special cells. This does not hold in the conch, for the fluid is secreted from the entire surface of the retina, as we shall presently show.

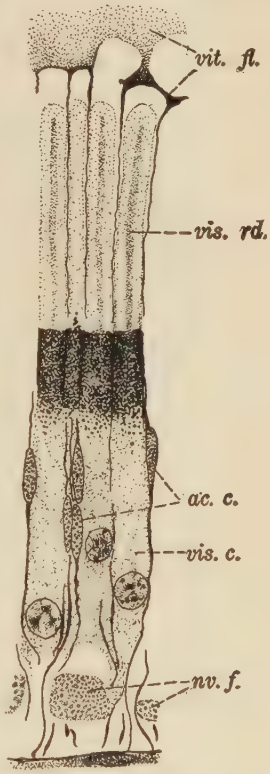

FIG. 218. — Enlarged portion of retina of *Strombus gigas*. *vis.c.*, visual cell whose visual rod (*vis.rd.*) projects above from the distal surface of the pigment layer; *ac.c.*, nucleus of accessory cell which makes and operates the pigment and also forms the vitreous fluid; *vit.fl.*, vitreous fluid which emerges in thin streams from between the visual rods; *nv.f.*, nerve fiber bundles. × 920.

The visual cells form the largest part of this retina, which is wonderfully clear and easy to study (Fig. 218). They extend from the nerve

bundles near the outer basement membrane to a point one half the thickness of the retina, where they stop and are continued distally as the visual rods. Between the visual cells are found the sustentacular cells, which extend as thin fibrillar bodies from the basal membrane up to the tops of the visual cells. Here they expand into a pigmented cytoplasm, which surrounds and lies between the ends of the visual cells. The nuclei of the visual cells are round and full, while those of the sustentacular cells are very much smaller and elongated.

The visual rods are nearly as wide and as long as the cells from which they come. Passing down their center almost to the tip is an axial filament, which is of considerable thickness and is fibrillar. The ends of the rods are frequently shrunken by the process of preparation, but, when seen to advantage, they are full and rounded and almost touch the vitreous body. The material of this body extends, as very regularly arranged threads, up between the rods as far as the pigment layer, where it is lost. The writers believe that these threads represent a flow of secretion from some cells in the retina to supply the growing vitreous body. Just which cells produce this secretion was not determined. Judging from what is known of other worms, it must be the pigment cells.

The nerve fibers form series of bundles lying near the basal membrane. They are seen cut in transection in Figure 218. Also one of the visual cells may be seen sending a process into the bundles. The basal membrane is very well marked both in form and staining power. It is lined externally by thin flat cells, one of whose nuclei is to be seen in the figure.

In all the lower mollusk forms the sensory cells possess much the same kind of visual rod or rhabdome. The rhabdome is varied to a rather more elaborate and fan-shaped rod in *Helix*.

There remain yet **two very complex mollusk eyes** to be described, that which is found on the mantle edge or back of many pleeypod mollusks as *Pecten*, *Spondylus*, and *Oncidium*, and that of the dibranchiate Cephalopods. These are most complex in structure and probably the most efficient of the eyes of invertebrate animals.

The eyes of *Pecten* are found scattered on the edge of its mantle folds on short stalks, or lying directly on the surface. Among the former is the eye of *Pecten irradians*, a common American form found on the eastern coast of the United States.

The entire eye is covered with a simple epithelium bearing a very insignificant cuticle and resting on a well-developed basement membrane. Most of these cells are tall and are heavily pigmented in their proximal ends, the nucleus lying midway in the cell at the point where the pigment ceases. The area forms a very broad band about the equa-

torial region, leaving a somewhat thinner and very transparent pole of the eye for the light-rays to come through. In the corneal cells the nucleus is placed somewhat higher.

The place inside of this outer covering contains the remaining organs of the eye in the following order from distal to proximal positions. A lens, a blood sinus, the several layers of the retina, a space filled with a vitreous humor, the argentea, and the pigmented tapetum. These all lie in an oval space, the eye-sac, and a nerve approaches this sac through

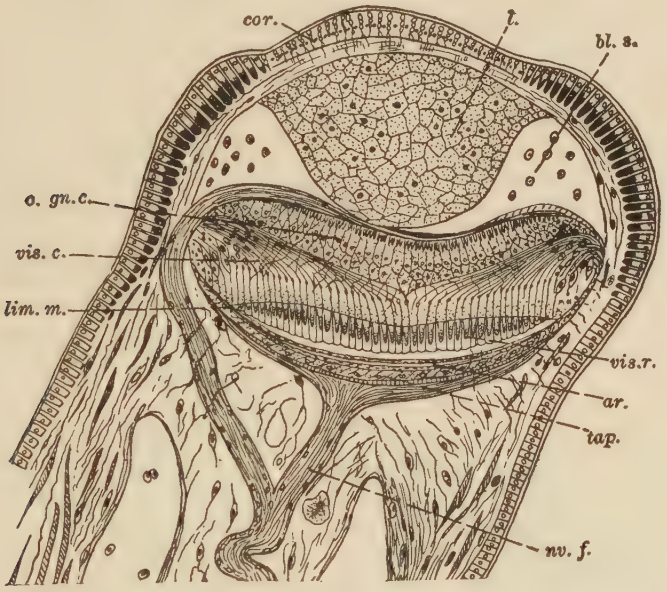

FIG. 219. — Eye of *Pecten irradians*. *cor.*, cornea; *l.*, lens; *bl.s.*, blood sinus; *o.gn.c.*, outer ganglion cells upon which lies a layer of supplying nerve fibers; *vis.c.*, visual cells; *lim.m.*, limiting membrane; *vis.r.*, visual rods; *ar.*, argentea; *tap.*, tapetum; *nv.f.*, nerve fiber. (After PATTEN.)

the proximal connective tissue and divides into two branches, a basal branch and a lateral branch.

The lens is unlike so many of the lenses we have previously examined, in that it is composed of a mass of cell bodies instead of secreted cell-products. These cells have round nuclei of moderate size, and are nearly fitted together to form a round body with curved surfaces coming to a sharp, circular edge. This body lies directly against the cornea whose proximal surface is in contact with its distal surface, and its edges reach as far as the pigment area of the cornea or iris. Its proximal curve, projecting into the blood space, almost touches the next organ, which is the retina (Fig. 219).

This retina is a thick mass of tissue inclosed in a separate membrane

and reaching across the eye-sac, so that it separates the blood sinus in front from the vitreous fluid behind. Its most important layer is the layer of visual cells, and these are found on its proximal surface pointing backward. This makes the eye an inverted form like that of the spider and that of the scorpion. As we shall see later, this peculiarity is also true of the human eye.

The visual cells form a thick layer and are rather peculiarly arranged. Their distal ends give off the long, heavy visual rods, each of which contains an axial filament. Hesse describes two filaments in an occasional rod of *Pecten tigrinus*. These rods form a very even and thick layer in the section. The line at which they all take their origin from the visual cells is straight and even, and marked by a set of fine plates in the substance between the cells. These plates are parts of a large, continuous membrane, the *limiting membrane*, which has many openings for the rods to pass through. Miss Hyde has described these rods as separate cells beginning at and separated from the visual cells by the limiting membrane, and each with a nucleus of its own. Hesse does not find this nucleus in several other species of *Pecten*, and in *Spondylus*, and the writers could not find it in *Pecten tenuicostus*.

The visual cells come from the sides of the retina, and by well-graduated curves turn in a proximal course until they end on the limiting membrane, where the rods are given off. Their nuclei are large and oval, and lying among them are a number of slender sustentacular cells with long, thin nuclei. These supporting elements give off fibers which seem to pass outward and reach the layer of the cells above them.

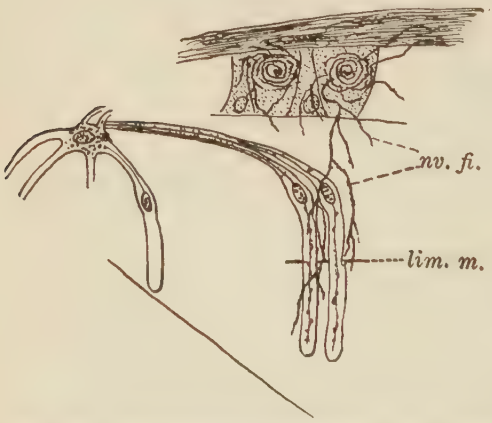

FIG. 220. — Two outer ganglion cells and three visual cells from the retina of *Pecten irradians* to show their connection through nerve fibrils, *nv. fi.* These fibrils form a spiral coil around the nuclei of the outer ganglion cells, and pass into the main visual cells. *lim.m.*, limiting membrane. (Modified from Miss HYDE.)

Lying still outside the layer of visual cells is a single layer of stout, heavy cells with large nuclei. They are called the outer ganglion cells. This layer shows a slight differentiation in ordinary preparations, and under proper methylene-blue treatment it is seen that the row as seen in section is composed of alternate nerve and supporting cells. The methylene blue shows that neuro-fibrils enter this layer from the lateral

nerve branch, and that these or others wind in a spiral through the cytoplasm around the nucleus of these nerve cells, and then pass out inwardly to send branching ends to the visual cells (Fig. 220). Miss Hyde does not show an outer (one cannot say as yet "proximal" or "distal") area of cytoplasm on these *external ganglion cells*, as Hesse and others have pictured, in an analogous position, in so many other pecten eyes and as the writers saw in *Pecten tenuicostus*. Some faint indication of it is shown in one of her figures.

This edge shows that the cells of this layer have some very peculiar nervous function besides their connection with the visual cells as described by Miss Hyde and as weakly shown by certain fibrils in the figures of Patten and Hesse. The edge is drawn out into thick groups of rod-like processes, which converge toward an outside central point and join the upper branches of the optic nerve. In doing this, it can be seen, they come to act as intermediate cells between the visual cells and the central ganglion. These visual cells also have a direct connection by means of the lower or lateral branches of this nerve, so that there are two different pathways for the impulse, and we have a case where communicatory nerve cells have entered the retina as they had begun in the worm, *Vanadis*, where they formed a ganglion below the retina and outside of the eye. This layer of cells probably has some function to perform which is analogous to that of the layers of ganglion cells in the eye-stalk of the Arthropoda or the ganglion layers of the vertebrate retina.

Beneath the retina, and separated from it by the wide space filled with the vitreous fluid, is the *tapetum*, a layer filled with refracting granules and used apparently to reflect the light. This layer is formed by a single, wide, thin cell in other pectens, and the same will probably be found true in the form we are studying. Underneath the tapetum is the pigment layer, a simple epithelial covering whose thick cubical cells are filled with red pigment granules.

The dibranchiate cephalopod eye is probably one of the most complex in existence, although this complexity is more easily understood and more superficial than in the vertebrate eye. The eyes of both of these groups should be studied from the developmental point of view to properly understand their histology.

The eye of the squid, *Loligo Pealii*, begins as an invagination which, as in so many other mollusks, becomes the visual sac (Fig. 221). At an early period this sac becomes cut off from the exterior, and develops the posterior part of its lining epithelium into the visual epithelium. The anterior region develops on its inner surface a rather larger part than one half of the lens. This lens is a homogeneous cell-product which is formed as a series of very perfect lamellæ lying parallel to each other. The ectodermal surface, which faces *outward* from this inner lens epi-

thelium, forms the other part of the lens as a distally directed and supplementary portion of the whole outline of the lens, which is almost spherical. Thus the surface epithelium, which in other mollusks, as *Strombus*, is used to form the cornea, in this case forms the outer part of the lens; while the inner surface, which forms the entire lens of *Strombus* and other higher mollusks, in *Loligo* forms the proximal two thirds of the lens.

It will be remembered how, in most of the previously described mollusks' eyes, an area of pigment was placed about the cornea to keep

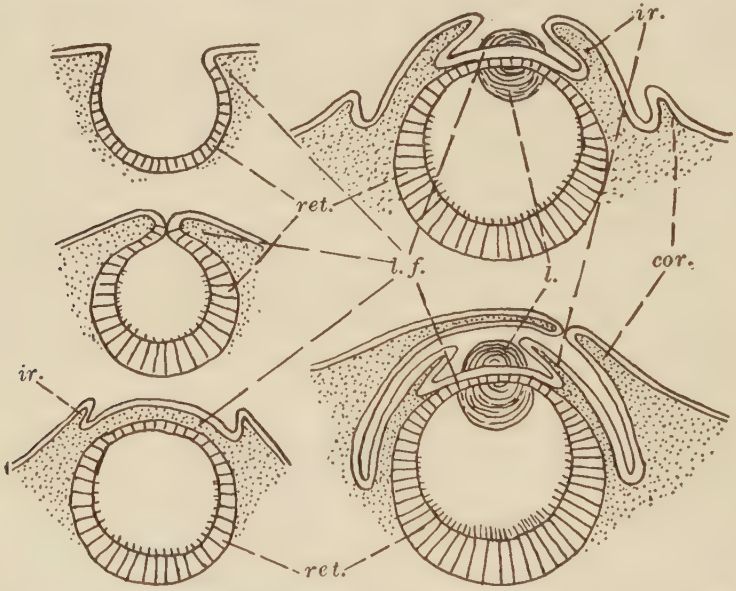

FIG. 221.—Five outline sketches to illustrate the histogenesis of the eye in a dibranchiate cephalopod. *ret.*, retinal epithelium; *l.f.*, fold which forms the lens; *l.*, lens; *ir.*, iris fold; *cor.*, corneal fold. (After LANG.)

too much light or too oblique rays of light from entering the eye-sac. This same region of pigmented epithelium is found in *Loligo*, but it has been raised into a circular ridge, and then this ridge is drawn centrally as a circular septum, with a central aperture for the light to pass through. The amount of light to be admitted is thus determined by the septum, which can enlarge or diminish the central opening. It thus becomes a very perfect form of *iris*.

Still a third ridge of integument, lying outside of the iris, is now developed and closed in centrally until it forms an external covering for all the other structures. This is the cornea which, it can be seen, is not homologous with that of any other mollusk. The tissues over the eye are transparent, and are composed of an outer and inner epithelium with

a small amount of connective tissue between. The corneal fold never entirely closes up in many cephalopods, but leaves a minute water canal. Thus the corneal cavity is filled with sea water, to which is possibly added some secretion of the neighboring cells to keep this water free from parasites.

A few histological details should be explained before we proceed to the more important retina. The tissues back of the primary eye-sac become much specialized. They are mostly of various connective-tissue

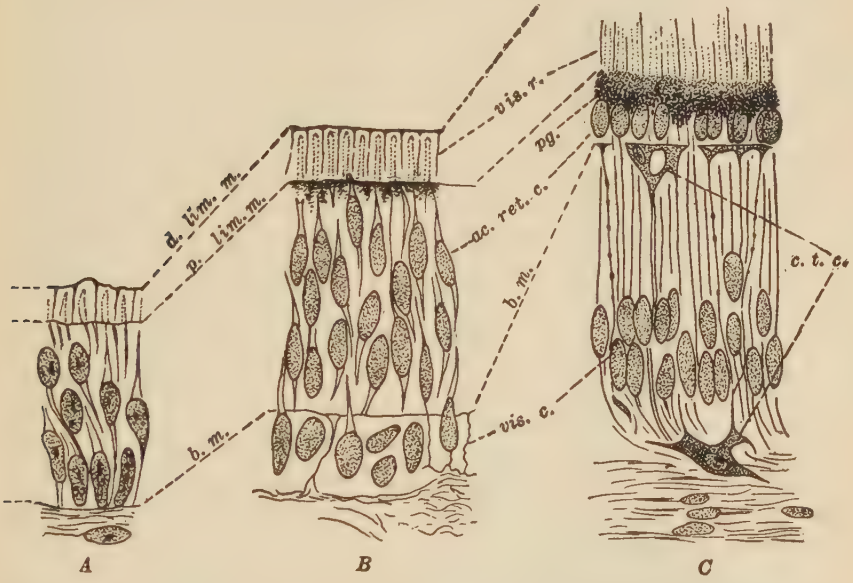

FIG. 222.—*A*, *B*, *C*. Three stages in the histogenesis of the retina in the cephalopod mollusks (*A*, *B*, from *Sepia*; *C*, from *Eledone*); *b.m.*, basement membrane, below which the nucleated bodies of the visual cells have migrated in *B*, *C*; *p.lim.m.*, proximal limiting membrane; *d.lim.m.*, distal limiting membrane; *vis.c.*, visual cells; *ac.ret.c.*, accessory retinal cells; *c.t.c.*, connective-tissue cells, one of which contains an intra-cellular blood channel; *pg.*, pigment in the distal cytoplasm of the accessory retinal cells; *vis.r.*, visual rods. (After HESSE.) × 600.

forms, and some develop into smooth muscles to move the eye to a small degree. A cartilaginous capsule is formed so that it incloses most of the eye-sac, and is provided with many perforations through which the nerve fibers pass from the retina to the huge optic lobe of the brain. This lobe is usually in very close contact with the eye-sac. The double layer of epithelium which forms the two parts of the lens is an interesting study, for the details of which we have no space; also the rigid connective-tissue elements on the outside of the eye-sac and iris.

The retina is naturally the most important tissue. It begins, as in other mollusks, as a columnar epithelium, whose elements lengthen until

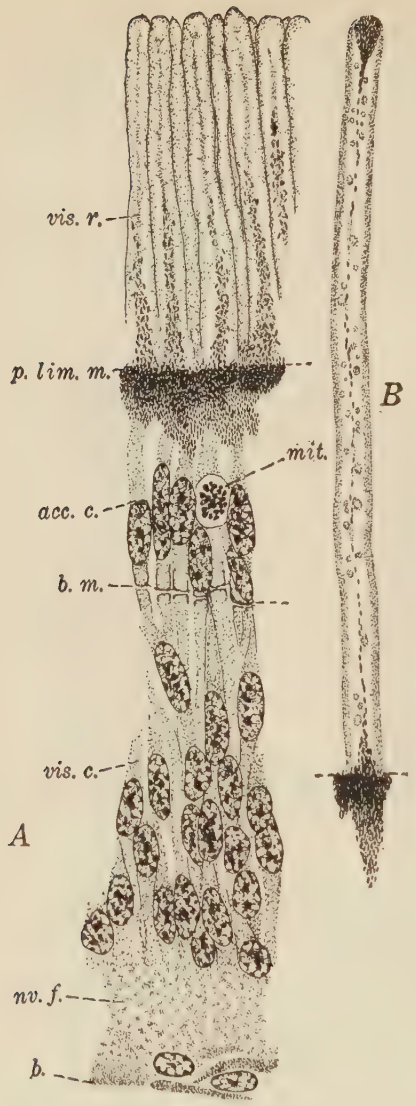

FIG. 223. — *A*, bit of retina from the eye of a 12 mm. squid, *Loligo Pealii; p.lim.m.*, proximal limiting membrane; *b.m.*, basal membrane (now situated above the real base at *b.*); *vis.c.*, visual cell bodies; *acc.c.*, accessory cell bodies; *vis.r.*, visual rods showing the pigment ascending halfway, about the central axis; *nv.f.*, nerve fibers forming basal layer; *mit.*, mitosis in accessory cell; *B*, visual rod from a visual cell of a half-grown *Octopus Americanum*. The animal was out in full tropical sunlight for an hour or two before being thrown into a strong fixative, and the pigment has ascended around the central axis and collected as a knob at the distal end. × 920.

they appear, as in Figure 222, *A*, resting proximally on a basal membrane. At this time some of them begin to form a layer of visual rods on their distal ends. The rod layer is marked off from the cells by a slight membrane, the limiting membrane.

Figure 222, *B*, shows the next stage in development, which consists in a proximal migration through the basal membrane of all the rod-bearing or visual cells, leaving a single row of their neighbors distal of this boundary (Fig. 222, *C*). These latter cells do not function as supporting elements, but are used to produce and operate two materials: a brown pigment to be used as a protecting pigment, and a fluid which is conducted distally between the rods and forms the *outer* or *distal limiting membrane.*

This membrane is of considerably greater thickness in some forms than in others, and is exactly homologous with the fluid, vitreous body which was seen in the eye of *Strombus.* This latter, too, was doubtless formed by the sustentacular cells found in the retina, and was delivered into the eye-sac lumen as the threads we saw passing distally between the visual rods. These cells, which we shall call *accessory retinal cells* instead of the "limiting cells" of other writers, are in contact proximally with pro-

cesses from connective-tissue cells which lie far below the basal membrane and the visual cells. These processes often contain intra-cellular blood capillaries, and sometimes the connective-tissue nucleus is found in them. The line of contact between an accessory cell and such a connective-tissue process is always on the basal membrane.

The visual cells in the adult have their principal cell body and nucleus far below the basement membrane (Fig. 223, *A*, *B*). The proximal end of each is produced into a nerve fiber, and the distal end, just above the accessory retinal cells, gives rise to a long, wide, and round-ended visual rod. The most important structure in the eye is the neuro-fibril, which enters the cell body and passes through the cell to enter into the visual rod as an axial filament. Here it traverses the whole length of the rod in a somewhat sinuous course and terminates as a knob in the tip. The relations of the pigmented cytoplasm of the accessory retinal cells to this axial filament and end-knob are most interesting. When in the dark, the pigment is all to be found in the pigment band, which marks the distal ends of the accessory cells. As the light increases, the pigment-bearing cytoplasm of these cells travels in a thin stream that surrounds the fibril until it reaches the knob (Fig. 223, *A*, represents an intermediate stage). When the light is brighter, as in direct sunlight, the knob itself is surrounded and appears as a brown lump. This last condition is seen in a single rod from *Octopus*, pictured as Figure 223, *B*.

We should speak here of **the remarkable condition found in the tetrabranch cephalopods**. The eye of *nautilus* is much like the squid's as to retina, but all the complex accessory apparatus is wanting. No lens or cornea is present, and yet the well-developed retina has an image projected upon it by the way its mouth of invagination is arranged as a *pin-hole camera*. Through this hole the image is formed as in a *camera lucida*. The eye-sac is full of sea water and in constant communication with the water.

As *Nautilus* is a more rudimentary form, it may be asked if this represents a more primative condition. It may, as it is analogous at least to such eyes as those of *Patella* and *Trochus*. But we must remember that the surviving members of ancient races frequently show marked degenerations which amount to simplifications, and it is quite possible that *Nautilus* shows such a condition in its eye. Its retina suggests the latter.

The mammalian eye, which represents to a degree all vertebrate eyes, is not only as complex histologically as the cephalopod eye, but it is more highly organized and is probably the most efficient eye in existence. In its derivation from the embryonic tissues it is unique, and we shall understand it best by studying it from that point of view.

The most distinctive feature of its development consists in the fact that two principal tissues take part in forming it jointly. The retinal

layer, instead of being invaginated from the site of the future eye, is invaginated from a wall of the neural tube which lies under the site of the future eyes; thus it is seen that the retina is a part of the brain's wall (Fig. 224, *A*). It must be remembered in this connection that the brain tube was itself an invagination of the original ectoderm.

The neural invagination reaches toward the skin in a cup-like form, and at the same time a thickened area of this skin forms a depression

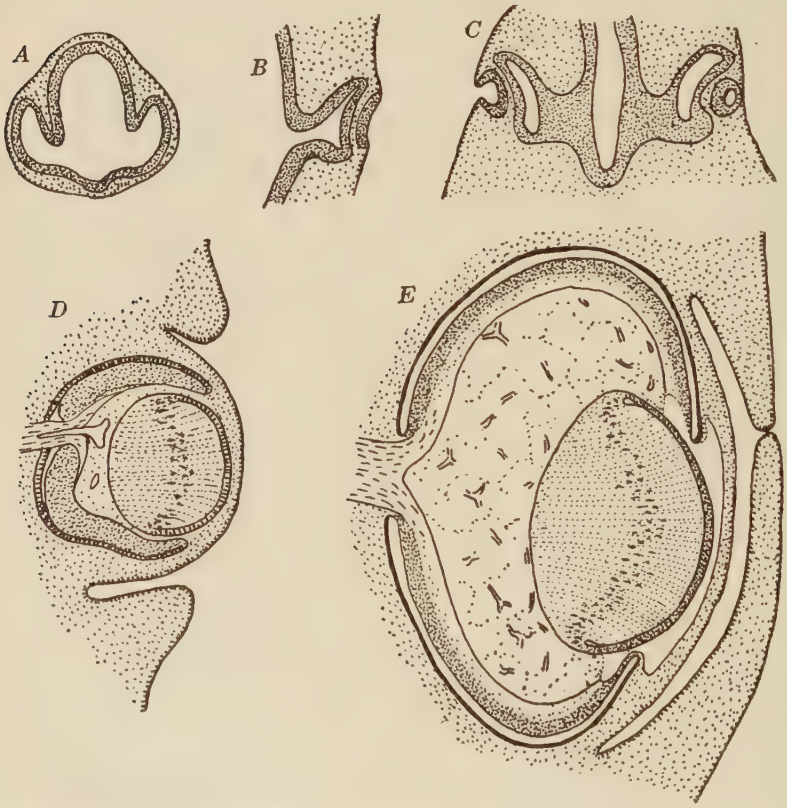

FIG. 224. — Five sketches to represent five stages in the development of the rabbit's eye. (From "STÖHR'S Text-book of Histology" by LEWIS.)

which advances inward to meet it (Fig. 224, *B*). This latter structure becomes the lens by being constricted off as a sac from the external epithelium (Fig. 224, *C*), and undergoing a thickening of its posterior wall of epithelium, until this wall fills the sac up solid with its long, parallel, fiber-like cells. Its nuclei thus form a row across the middle of a section of the lens (Fig. 224, *D*, *E*), and the anterior wall becomes a layer of simple epithelium covering the anterior surface.

The edges of the *optic cup*, as the brain invagination is called, embrace

and hold the lens in place (Fig. 224, *D*). The rear wall of the optic vesicle becomes a pigment layer, and the anterior or external wall becomes the visual layer, which is much complicated by the differentiation of communicatory nerve cells as well as sustentacular cells out of its epithelium.

The corneal layer become separated from the lens by a space containing a fluid, the *aqueous fluid* (Fig. 224, *E*). The space behind the lens and between it and the retina is filled with the vitreous body. This material has its origin in the connective tissue which occupies the space when the optic cup is first formed. It develops blood vessels and a bounding membrane which persists, while the blood vessels and part of the connective tissue atrophy, leaving the space filled with a fluid and a few cells. The cornea is lined outwardly by a clear, stratified epithelium of some thickness and two principal layers. It rests on a thick basal membrane. Under this is found the thick, strong connective tissue which is laminated and composed of fine fibrils of connective tissue with long, flat nuclei. Inwardly is found a somewhat thinner basal membrane on which lies a thin and single-layered epithelium which is in contact with the fluid that fills the anterior chamber of the eye.

The lids are two folds which arise from evaginations and are analogous to the squid's cornea, while the vertebrate cornea would have to be compared superficially, as to its origin, with the squid's iris. Glands are found in the lids, and a large compound tubular gland, the lachrymal gland, is placed near the eye to pour a lubricating, cleansing, and moistening fluid out upon its corneal surface.

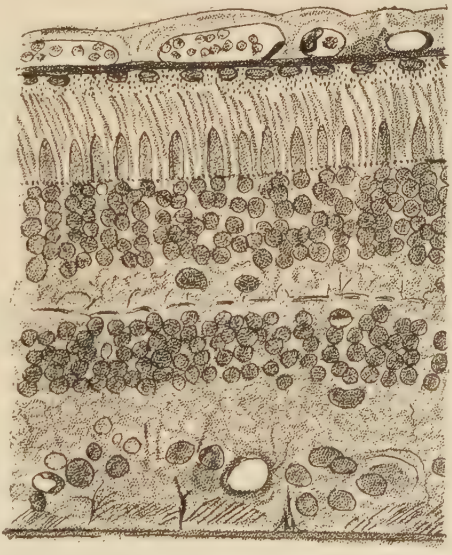

FIG. 225.—Section to show the apparent layers of the human retina. *nv. f. l.*, nerve fiber layer; *g. c. l.*, ganglion cell layer; *in. n. l.*, inner nuclear layer; *H. fi. l.*, Henle's fibrous layer; *o. nu. l.*, outer nuclear layer; *r.* & *c.*, rods and cones; *pg.*, single layer of pigment cells; *bl. v.*, blood vessels. (From "STÖHR'S Text-book of Histology" by LEWIS.)

The retina is the most interesting layer and is very complex. We shall study it by describing or, more properly, enumerating its various apparent layers in order, and then explaining the meaning of some of them as

indicated by the special processes of staining (Fig. 225). The innermost layer, which is a distal region when explained embryologically, is a layer of nerve fibers passing in many directions and all terminating at the *blind spot*, where they dip down through all the other layers to become the optic nerve. Just under this layer is found a scattered layer of ganglion cells, which form the *ganglion-cell layer*. This last is followed by a thicker fibrous layer termed the *inner nuclear layer*. This is composed of many small, round nuclei about five deep. On its outer margin comes the thin *inner reticular layer*, and this is marked off from the thicker *Henle's fiber layer* by an incomplete membrane.

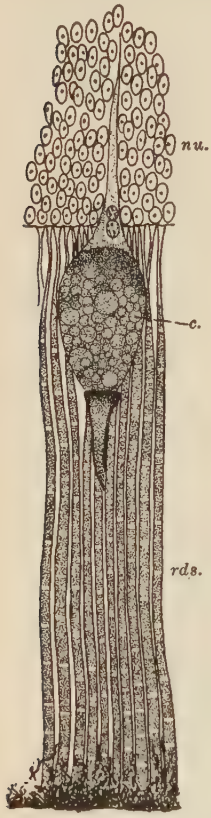

FIG. 226. — Part of the retina of a herring, showing the two kinds of cell-organs by which the visual cells perceive light. Ten rods (*rds.*) and one cone (*c.*). *nu.*, nuclei of the rod cells. Two nuclei visible in the cone cell. × 1000.

Next to Henle's layer is found the *outer nuclear layer*, which is like the inner except that it is thicker, being about six or seven nuclei deep. Its outer side is marked by the sharp and clear *outer limiting membrane*. From this membrane the rods and cones of the eye project distally as a thicker layer. They are both of equal height, and each is composed of two segments. The rod segments are about equal in length and show no great differentiation. With the cone it is different, for the basal segment is of huge cone-shaped bulk, many times as thick as the rods; while the distal segment is very much like that of the rod.

The rods and cones are particularly well seen in some fish, as in the herring. Figure 226 represents a cone and several rods from this fish. The cone is not as high proportionally as in man, and part of the outer segment has been cut off in preparing the section. This preparation is particularly happy in showing the transverse lamellæ of which the rods are composed. The rods and cones form the distal layer of this wall of the original optic cup, or eye-sac. The opposite wall, however, has been closed in and intimately applied to them, so that it appears to be and is indeed another layer of the retina. It is a simple layer of cuboidal cells, filled with pigment in which the distal ends of the rods are buried. When the light becomes too bright, the cytoplasm of these cells is pushed up between the rods, and thus it partially shades them. This is a very different condition from that found in many other eyes.

Some explanation of the real relations existing between these different layers is highly necessary. Figure 227 is a diagram which explains in part what has already been discovered. As in other retinas the rods and cones are the visual cell-organs. The visual cells themselves form a thick layer below the external limiting membrane, and their nuclei form the *outer nuclear layer*. The bodies of the cone cells are thicker than those of the rod cells, and their bases are more greatly expanded. The line of these bases is the membrane-like line between the inner reticular layer and Henle's layer of fibers.

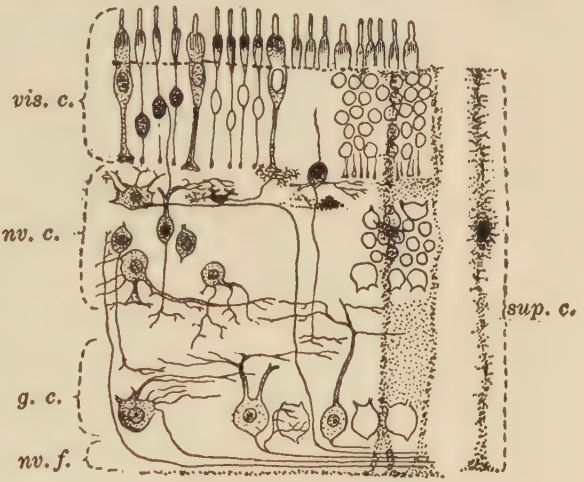

FIG. 227. — Diagram of some of the known elements of the retina in man. Compare with Fig. 225. *vis.c.*, layer of visual cells whose perceptory organs form the rod and cone layer and whose nuclei and processes form the outer nuclear layer and Henle's layer; *nv.c.*, layer of bipolar and amakrine nerve cells whose nuclei form the inner nuclear in ordinary preparations; *g.c.*, ganglion cells of the ganglion cell layer; *nv.f.*, nerve fiber layer; *sup.c.*, supporting or neuroglia cells. (From "STÖHR'S Text-book of Histology" by LEWIS.)

The other cellular layers represent a number of different kinds of nerve cells whose processes make various intricate connections. The use of these connections is obscure and not really known. All that can be said is that the various cells act in some way together and correlate the various impulses in a way that prepares them to represent the image to the brain. Some of these nerve-cell relations are shown in the diagram represented by Figure 227.

There are sustentacular elements in the retina of man which extend from the fiber layer to the rods and cones, and serve to hold all together. These are known as the radial fibers, and two of them are pictured in the diagram. They are neuroglia elements.

The connective tissue, muscle, and other histological details of the outer parts of the eyeball as well as of the iris and sclera cannot be taken up here. Many blood vessels penetrate all parts of the eye, including the retina, to supply its various needs.

Technic. — Eye preparation presents the greatest possible variety of technic, owing to the very great variety in the different kinds of eyes.

The nerve elements demand the skillful use of silver and methylene blue. The combined maceration and section method outlined in the last chapter is useful in studying the retina and visual epithelia of many eyes. For the more general histological relations the ordinary paraffin sections will do when the eye is one of the simple ones whose tissues are homogeneous. In perhaps the majority of cases, however, the lens acts as a formidable obstacle to the securing of perfect or even fair sections. The lens can seldom be softened, and the best way is to remove it by careful dissection after the tissue is fixed and before the embedding has been begun. With the lens removed very good general sections can be cut, especially in celloidin, in which case, however, the sections are apt to be too thick. In the arthropod eye the difficulty is not the lens but the cuticle, and this is not so formidable an obstacle. The cuticle can sometimes be removed, especially when it is very thick. At other times it can be cut in the case of a thin-shelled or newly moulted animal. For general studies of the tissues Zenker's fluid or chrom-aceto-formol is the best. For a good picture of the retina Flemming's strong fixative or some other fluid containing osmic acid is the best.

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THE OLFACTORY AND GUSTATORY NERVE TISSUES

The olfactory and gustatory nerve cells form a comparatively small group of perceptory neurons, which are distinguished from the others by the fact that they can receive impressions directly from the atoms and molecules of some substances that have been dissolved in the atmosphere

or in water. These impressions are such as distinguish the quality or individuality of the substance, and only a certain proportion of known substances can produce an impression, at least in the case of man. Many different substances produce similar impressions, and would be identified as the same by most animals.

The power is one that we do not fully understand, partly because our own organs of taste and smell are so poorly developed. The delicacy of this perception in other animals is wonderful beyond belief, while in still others it is very poorly developed or not at all. In the case of man it is probably degenerate from a former condition of high efficiency.

The cells that perform the function are of necessity epithelial in character, and in the cases where we have seen them they are usually very thin and elongate. There is no peculiar structure of their sensory ends by which they can always be distinguished from some other perceptory cells of the simpler types that we know certainly to be tactile or other cells. In both we meet with a variety of rod- or hair-like perceptory organs. In all cases the function must be determined either by experience, which we can only do in man; or by homology, as is done in the other classes of vertebrates; or by experiment and observation, as must be done in all other animals. Thus we cannot be sure of the exact function of many of the organs that have been given the name of "olfactory" or "gustatory" organs in a large number of lower forms.

In the vertebrates we find the gustatory and olfactory cells forming two distinct types, the only strong bond between them being the fact that they are both used to perceive chemical qualities.

The olfactory cells are the more typical of the two and so similar in all forms to those of the birds that we shall study them as found in the common fowl, comparing them with the well-known form of man. **The epithelium on the olfactory prominence of the chicken** (Fig. 228) consists of the same three sorts of cells found in the mammals, a sustentacular group, the more numerous in number, and a liberal number of olfactory cells lying scattered among them, the whole resting on a layer of basal cells. A basement membrane is so weakly developed as to be apparently absent.

The sustentacular or supporting cells have long bodies reaching from the surface down to the peculiar layer of basal cells. Here the proximal end of the cell branches once or twice, and its ends are attached to and intermingled with the processes of the basal cells. The cell body is of an irregular form, expanded to contain the nucleus somewhere in its distal two thirds, and it does not show the strong granular differentiation of proximal and distal cytoplasm that these cells do in the human tissues, except that the distal end secretes mucus in small quantities, and in some cases is apparently ciliated. False appearances of ciliation are to be

seen in parts of the mucus that covers the epithelium, as is also the case in the mammals. The distal ends of the sustentacular cells form an even surface. The pigment that gives the characteristic yellow or brown color to this surface in so many forms of vertebrates is here deposited as a thin layer in the outermost parts of these cells. The nuclei are easily distinguished from the nuclei of olfactory cells by an oval form and smaller size as well as a different chromatin pattern. They lie in a broad zone of the epithelium composed of the distal two thirds of the cells, and are therefore to be found at varying heights in the cell. In the

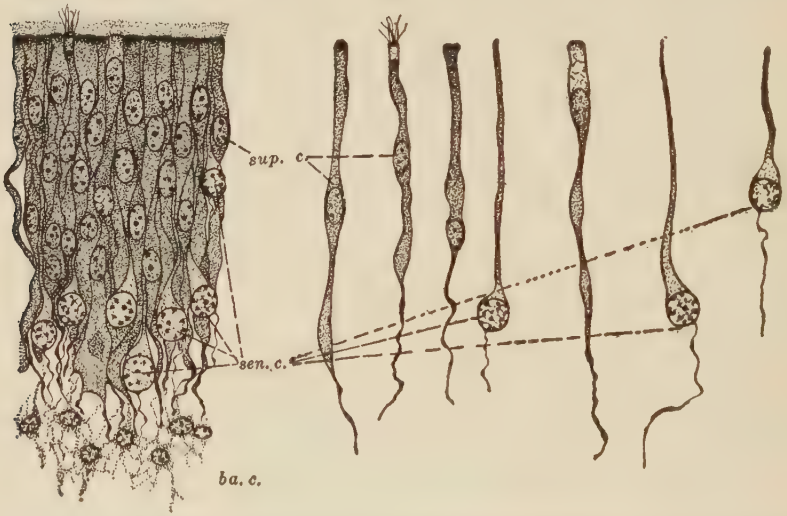

FIG. 228. — Bit of olfactory epithelium from the fowl, *Gallus domesticus*. Seen *in situ* to left. Several individual cells shown separately to right. *sup.c.*, supporting cells; *sen.c.*, sensory cells; *ba.c.*, basal cells.

mammals they are almost always found at one height in the cell, and therefore lie in a single plane and appear as a single row in section.

The third kind of cells in this tissue, the basal cells, are ectodermal in origin, and therefore an integral part of the epithelium. In the fowl this fact does not appear to advantage, the cells looking as much like branching connective-tissue cells as anything else. This appearance is intensified by the lack of a clearly defined basement membrane. The cytoplasm of the basal cells is granular and branching and lacks definite boundaries. It forms a reticulum with the cytoplasm of its neighbors, and the other epithelial elements rest upon it. In man the basal cells lie in a very much narrower row between the bases of the supporting and olfactory cells.

The olfactory cells are to be seen lying between the sustentacular

cells, with their round, clearer, nucleated bodies below the level of the lowest nuclei of the sustentacular cells. The well-rounded nuclei almost fill this cell body (Fig. 228, *sen. c*). Some few have the body up in the outer third of the epithelium. They give the lower third of the epithelium a distinctly lighter appearance.

The cell body is drawn out distally into a thin, smooth process that reaches to the surface and acts as the perceptory organ. Perceptory rods or hairs are not to be distinguished. The perceptory process is thinner than the distal processes of the sustentacular cells, but not so thin as the proximal portion of the cell to which it belongs, which is drawn out into a much thinner and longer process, the centripetal nerve fiber. This fiber, which is never myelinated, passes entirely out of the epithelium, and running through connective tissue and bone, it ends in the central nervous system, as in man, in the olfactory bulb. Here the fibers, in mammals, end in peculiar round bodies known as the glomeruli. These are not cellular structures, but are formed by the branching ends of the fibers from the olfactory cells united with similar branching end-organs from nerve cells in the bulb, the mitral cells, and others. Figure 229 shows these relations by means of the silver process used on an embryonic *Tropidonotus*.

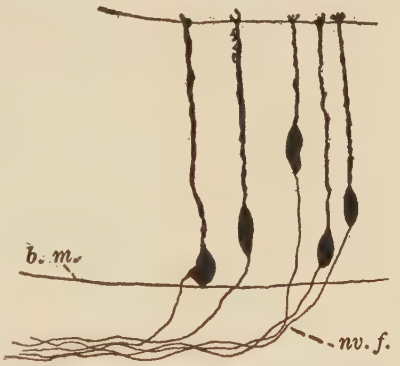

FIG. 229. — Silver picture of the olfactory cells in the nasal epithelium of *Tropidonotus*. *b.m.*, basement membrane; *nv. f.*, nerve fibers. (After RETZIUS.)

The relation of the olfactory cell to the second kind of chemico-perceptory cell that we shall study, the gustatory cell, or cell of taste, is a close one as regards function. In regard to its origin and structure, however, it may be strongly contrasted with it. Both perform the same function, but do so in different ways and attain different results. The olfactory cells deal with the finely divided atoms or molecules of substances in a gaseous state and usually dissolved in air or water. The gustatory cells, at least those of man, require more crude masses of the substance and require an immediate contact with them. Also they most probably must always be dissolved in water or some fluid of which water is a part.

The result is less definitive and delicate from the stimulation of a gustatory cell. We cannot, in this way, detect fine flavors and aromas, as can be done from olfactory cells, but secure only a few coarse impressions as sweet, sour, bitter, the warmth impression of alcohol, and such

tastes. The "taste" of our food is largely composed of olfactory impressions from the nose.

In structure the specific cells of the gustatory tissues are very different, in the vertebrates, from those of the olfactory organs. Their discharging end is not the extremity of a process extending into the brain. Instead, the centripetal end of the cell body is rounded, and the receiving nerve cells in the nerve center send afferent nerve fibers to the gustatory cells to receive the stimulus from the cell body.

The gustatory cells are not found scattered among an epithelium as are the olfactory cells, but associated in small numbers with certain supporting cells that somewhat resemble them in shape. Both of these, in the vertebrates, may be looked upon as simple columnar epithelial elements that were prevented by their function from developing, along with the rest of a former simple epithelium, into the stratified form. These small collections of taste cells, with their supporting cells, form structures known as "taste buds" and are to be found in various parts of the mouth in the higher vertebrates; while in the Amphibians and fishes they enjoy a wider distribution, even out to the face and head or on fin-rays and barbles developed for this purpose. In man they are to be found on the vallate papillæ, the sides of the tongue, and on the outer surface of the epiglottis.

The specific cell of the tissue is a thin and elongated epithelial form reaching from the surface to the basement membrane where, unlike the olfactory cell, it ends. The cell is somewhat enlarged at its middle or lower part to contain the nucleus. Its distal end bears a short rod-like structure, the cell-organ of perception (Fig. 230). There is but little variation in the shape of this kind of cell throughout the vertebrate groups. One can see it thinner and longer in the skin of *Lamperta*, or shorter and thicker in the skin of several fishes. Its arrangements as a tissue with the accessory supporting cells is more interesting. The two together form isolated groups, with the specific cells tending to concentrate at the center of each group. A rather diffuse group of this kind is formed in the larval form of *Petromyzon*. In this case the thread-like taste cells are scattered

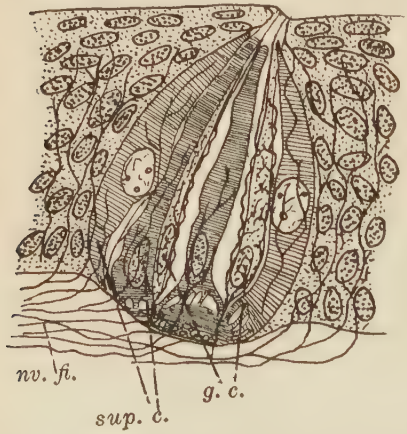

FIG. 230.—Taste bud in tongue of man. *g.c.*, gustatory cells; *sup.c.*, supporting cells; *nv.fi.*, nerve fibrils. From BÖHM and DAVIDOFF'S "Histology." (After HERMANN.)

among the heavy and far thicker supporting cells, which also have much larger nuclei. In most vertebrates the gustatory cells are few in number, and are collected into the central portion of the "bud," while the supporting cells surround it. In this position the wider or thicker middle portions of these cells, together with the narrow proximal ends and the still more narrow distal ends, make the whole organ oval in outline or melon-shaped.

The ends of the supporting cells are not arranged evenly with the surface of the surrounding epithelium, but form a depression or pit into which the gustatory rods of the taste cells project. The nerve supply comes as a series of afferent fibers from cells in or connected with the brain centers. These fibers enter the taste bud and end in lateral contact with the gustatory cells. The impressions received by the cells in their contact with the food or other substances are transmitted as impulses to these nerve ends which carry it to the nerve centers. In the mammalian taste organs some of the nerve endings enter the epithelium near the taste bud, but not in contact with the sense cells. While the real sensory endings are thicker and of irregular outline, these outer ones are thin and of smooth contour. They probably do not convey any gustatory sensation.

Another group of animals in which we can be almost certain the sense of smell and taste are present are the insects. These creatures have been proven by experiment to be able to smell much more keenly than man, and perhaps are better able to smell delicate and diffuse odors than the keen-scented mammals. A dog knows many individual persons by their smell, but an ant is able to distinguish all the members of its colony instantly by their smell and to make the power serve him almost as usefully as sight serves man. The carrion insects, beetles, blowflies, etc., can detect the presence of and find their way to any decaying protoplasm as certainly and quickly as many other mammals find their way to food by sight or a knowledge of its location. Among many forms of insects (as also some mammals) the females in the breeding season emit an odor that attracts the males from many miles away. This is particularly true of some night Lepidoptera. The sense of taste has also been proven by experiment to be present in many insect forms.

We shall first study the **olfactory tissues of insects.** These organs always are found on the antenna among certain possible tactile and other sense organs. The real olfactory organ is a hypodermis cell which has been differentiated into a nerve cell capable of being stimulated by certain odors (substances in a state of fine division in the air and, possibly, the water). This perceptory nerve cell has its proximal end (efferent end) drawn out into a nerve fiber which passes into the body to enter some ganglion.

The distal end of the cell is specialized into a distinct rod or cell-organ of smell. This rod may be clothed by a cuticular and hair-shaped cap, or it may be found at the bottom of a depression or " pit " whose object is to protect it. This pit is formed by the surrounding hypodermis, and is sometimes almost covered over on the top. The olfactory cell is sometimes very large and multinuclear.

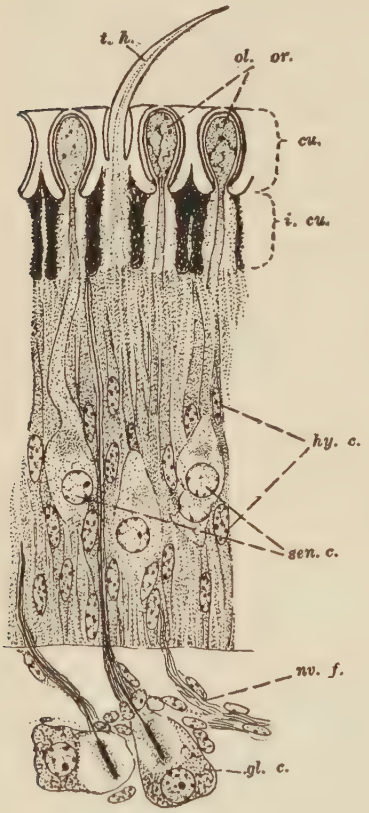

FIG. 231. — Olfactory epithelium from the antenna of a carrion beetle, *Necrophorus*. *cu.*, outer layer of the elaborate cuticle; *i.cu.*, inner layer of the same; *hy.c.*, hypodermal cells; *sen.c.*, sensory cells; *ol.or.*, olfactory perceptory organs of the sensory cells; *nv.f.*, nerve fibers coming from sensory cells; *t.h.*, tactile hair; *gl.c.*, gland cells, whose ducts pass through the olfactory epithelium to open through the cuticle. × 950.

The olfactory cells of the carrion beetle, *Necrophorus*. This insect is one of the first to arrive on the scene when an animal dies and begins to decay. The beetle has antennæ whose terminal joints are provided with parallel plates. The surfaces of these plates are lined with hypodermal cells that have built the greater part of the heavy chitinous wall. Among them lie the sense cells of smell, and some, perhaps, of touch also. Together, in our transverse section (Fig. 231), they form a very thick, apparently single, layer of epithelium, whose cells are so long and narrow that their exact boundaries cannot be easily determined. Occupying the center of the plates is a mass of connective-tissue cells, nerve fibers, and some large and very remarkable cells whose exact meaning is not clear.

The epithelium should first be studied. It shows plainly two kinds of cells. The thinnest is the wall-forming hypodermis cell, whose smaller and narrower oval nucleus shows a compact granular chromatin pattern.

The cuticle is peculiar. It may be said to have two layers. The outer layer is chitinous, but at many points it is depressed into deep pits. These pits do not open altogether through to the inside, but have their lower floor everted into the cavity so that it lines the pit as a second and very thin wall whose entire surface is perfect. Other, and less deep,

pits have this inner wall arising from a higher or more distal height and everted into a long, sharp, curved hair instead of the round, club-shaped organ seen in the deeper pit. The proximal layer is of a different kind of chitin and stains a different shade altogether, with most stains. It is not a layer in the proper sense of the word, being a set of proximal projections from the real chitin layer.

The nerve cells are readily recognized among the hypodermal cells by their thicker body and larger, clearer, and rounder nucleus. They lie with their main cell body in the proximal third of the layer, and thus their nuclei are found among those of the hypodermis cells. The cell body is drawn out distally and enters into one of the pit knobs or hairs. Both kinds are sensory, and knowing what we do about the tactile hairs of insects, it is fair to believe that the hair-covered endings are tactile, while the knob-covered endings are olfactory.

The knob-covered endings which rest in the deep pits are entirely homologous with the hairs. They are but shorter, blunter, and more deeply set hairs. They also exactly represent the " pegs " and other olfactory processes of Hauser, Graber, and many other writers. The nerve-cell cytoplasm which they contain is granular and represents a special cell organ of olfactory perception. It is aways protected from the exterior by the cuticle, and in no case were the writers able to find a case where the cytoplasm of either an olfactory pit, peg, knob or hair organ had direct access to the air. At first the broken and cut hair endings give this impression, but it soon becomes easy to detect the artifact.

The proximal end of the cell is drawn out into a nerve fiber, which runs in to connect with some ganglionic center. In connection with this nerve fiber one often sees a peculiar black line (in iron hæmatoxylin-stained specimens) which enters the epithelium and passes directly through it to the cuticle. Following this line back out of the epithelium, it is seen to reach one of the large cells mentioned above and enter into a vacuole-like area of this cell, where it ends in a cylindrical enlargement of some little length. These cells are large, with nuclei that are round and full and a cytoplasm that is granular, as in a nerve cell. At first sight they are liable to be taken for nerve cells, but must probably be considered as gland cells, and the line for a cuticular tube which ends in their vacuolar area much as the homologous tube does in the secretory cell of the odoriferous glands of *Belostoma*. The end cylinder is surrounded by a thicker covering of some less dense material. The perceptory or distal opening of the line tubule could not be made out. These very peculiar organs have been thought to be associated with the sense of smell, and the auditory and the static functions. They are probably, as far as we can determine for the present, cells that secrete

some fluid, of unknown function, which is discharged on the surface of the antenna plates. A variety of olfactory tissue in which the perceptory unit consists of an enlarged and multinucleated nerve cell has been described in several grasshoppers.

The organs of taste in insects have been very satisfactorily located experimentally, but they have not been differentiated structurally from the organ of smell. The palpi are used to taste with, especially on such insects as use them to touch food after it is in the mouth.

The mollusks also have sensory organs which can better, perhaps, be considered as olfactory rather than gustatory structures. They may

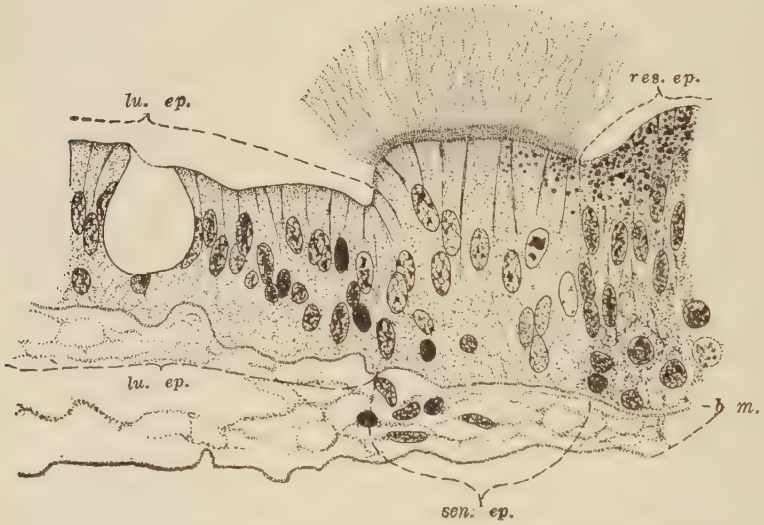

FIG. 232.—Central part, and epithelium on one side, of an osphradium plate of *Sycotypus canaliculatus*, transverse section. *b.m.*, basement membranes on each side; *sen.ep.*, sensory epithelium; *res.ep.*, part of respiratory epithelium; *lu.ep.*, portion of lubricating epithelium with one mucous cell. × 700.

not always be used to test or locate food matter, but are possibly of use in testing the purity of the water or other of its qualities which are of importance to the animal.

In their form, these cells are remarkably like the earlier stages of the olfactory cells in the vertebrate embryo, having their body drawn out into a short centrifugal process on which the sensory cell organ is developed; also into a long centripetal process, which acts as the nerve fiber to carry the impulse to the central ganglion. In regard to their tissue organization, we may find what appear to be two kinds of organs: one which is found as part of a modified gill, the *osphradium*, and another which is developed from the inner, mantle epithelium, in several places, in the cephalopod mollusks.

The osphradium of *Sycotypus canaliculatus* is a modified gill whose parallel plates show much likeness to the gill-plates represented in Chapter XVII. Figure 232 shows one side of a plate in vertical transection near its base. To the right is seen the beginning of the large extent of distal epithelium, which clothes the greater part of the plate. Its tall columnar cells hold heavy granules of pigment in their distal cytoplasm. The nuclei are at various proximal levels, and there seems to be a double layer of irregular basal cells with rounder nuclei. This epithelium is so like the gill epithelium that one must consider it as respiratory.

To the left and nearer the body of the animal is the layer of still less differentiated epithelium, which lines the deep curvature of the fold. It possesses some mucous cells, one of which is pictured, and its nuclei are comparatively higher or more distal in the cells than those of the outer epithelium.

Between the two, and placed in a longitudinal belt, comes the olfactory epithelium. The cells are longer and stouter, and their cytoplasm is clearer. The cilia represent the olfactory rods seen in many other gastropods, as *Segaretus*, where they are short and evidently not ciliated. Their length in *Sycotypus* seems to be rather an indication of low specialization. The body of the lamella and the epithelium on but one side of it are represented in Figure 232. The epithelium on the other side is symmetrical.

The peculiar organ, discovered by Van de Hooven in *Nautilus* and named after him, is considered to be an olfactory organ, or one for testing the water, as mentioned above. It consists of a region derived from the mantle cavity and lined with a thick, heavy epithelium. Where a section is teased and mounted, the same effect may be attained, as is shown in Figure 233. The large, heavy cells with proximal nuclei are gland cells and secrete a mucus.

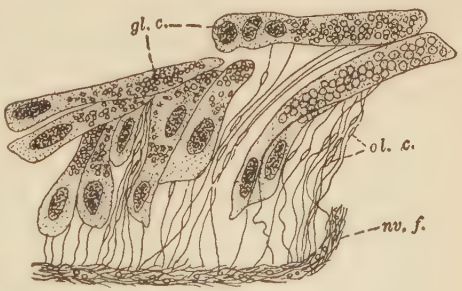

FIG. 233. — Teased preparation from the olfactory epithelium of a *Nautilus*. *gl.c.*, large gland cells which have been shaken out from among the thread-like olfactory cells, *ol.c.*; *nv.f.*, nerve fiber. (After GRIFFEN.)

Another set of cells, more numerous and much finer, lie between the gland cells and extend the same distance, from base to distal edge. These are the nerve or sensory cells, and they send efferent processes toward the central ganglia, while their afferent processes bear perceptory cell organs. The efferent processes form a heavy

layer of fibers in the connective tissue which lies just beneath the basement membrane. These perceptory cells are peculiar nerve elements because of the small size of their cytoplasmic bodies, which are barely large enough to hold the very small oval nucleus.

Technic.—Nitrate of silver by the rapid process and one good method of paraffin sectioning is all that is needed to study the olfactory organs, although the macerated section method is very applicable in this epithelium, also. Where the epithelium is covered with a cuticle, as in the insects, the cuticle is almost always very thin and forms no noticeable obstacle to the cutting.

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CHAPTER XIV

PIGMENT TISSUES

ANIMALS, in certain of their tissues and products of these tissues, are variously colored. These colors depend upon refractive surfaces, diffracting lines or markings, and inclosed colored substances called *pigments*. The various substances giving rise to the colors of the tissues and of their products are included under the term *pigments*. These pigments may be inert, serving merely as coloring and protective devices; or may be of great physiological importance. They occur either in a diffused or in a segregated form. Considered as coloring substances, they serve to protect from light or to conceal from the observation of enemies and prey and to attract or to warn other organisms.

Examples of diffused pigments among animal products are the bilirubin of the bile, the urochrome of the mammalian urine, the purple secretion of several nudibranch mollusks, and the brown or black secretion of the cephalopod mollusks. Other varieties, based upon their physiological use, might be demanded by physiologists in a complete classification, but the above must suffice as examples of what we mean by diffused pigments in tissue products. In general we shall consider here only such pigments as apparently exist to serve the animal by their color.

Many organisms have colors peculiar to them, which are due to diffused pigments in the tissues themselves. Among the Protozoa the members of the genus *Vampyrella* often present a diffused red pigment, which gives the specimens a characteristic color. Many of the colors of the insects are due to diffused pigments. Myochrome is a diffused pigment that gives the red color to mammalian muscle. Perhaps the most important diffused pigment is the hæmoglobin in the blood of all vertebrates and some invertebrates; in this case the pigment is a substance, the physiology of which we understand and in which the color is, possibly, more of an incident than a point of any importance to the economy of the organism. It can be said, however, that the color of blood does serve a distinct end. Other such diffused pigments of various colors are found in the blood of invertebrates. In many of

these cases the color does not appear until the blood is exposed to the air or to the action of other external conditions.

A more conspicuous form of pigment from an histological standpoint is that due to the segregation of the pigment material in the tissues or their products. In this condition the coloring matter is assembled in little granules within the cell, usually in the cytoplasm which it sometimes fills completely, and at other times it gathers in certain localities called the pigmented areas. These granules appear to play the same rôle for the animal pigments that the chloroplasts play for the chlorophyll in most plants.

The so-called stigmata of certain Protozoa are rather conspicuous bodies, which bear a bright red segregated pigment. In other Protozoa there are to be found granules that bear a brown pigment that is quite like that of the brown Algæ, as, for example, *Crysamœba;* other granules may be found that contain a dark to black pigment resembling greatly the melanin of higher forms (*Metopus*). In the invertebrates these dark pigment granules are very common. It remains to be seen if this resemblance to the melanin is superficial or a real one. Melanin is the most common form of the segregated pigments found among the higher vertebrates. It gives the characteristic color to the choroid of the eye and to the skin in the darker members of the human race. Other cells inside the body show it occasionally, as the nerve cells in age (see Fig. 163) and other cells in disease. In the nerve cells the granules may not be a true melanin, but if it is, its presence could be accounted for by the ectodermal origin of these cells in which the pigment was a vestigial character.

In the tissues of the lungs segregated pigments are found, which are important retainers of oxygen. Segregated pigments are characteristic of the tissues of the alimentary tract and of the liver. These are quite active as retainers of digestion products.

Diffused pigments, like urochrome, may be considered as excretions that have been taken up from the blood along with the other constituents of the urine. Bilirubin, on the other hand, is to be taken as a secretion of the liver cells. The pigment of the ink of the cephalopods is also a secretion. In the secretion of this matter the entire secreting cell is destroyed in the process. In certain Protozoa diffused pigments arise as direct products of alimentation. In *Vampyrella*, for instance, the red color of well-fed specimens is due to the color of the digested food held within the protoplasm of the cell. M. von Linden indicates that the red pigment of the intestine of the larva of *Vanessa* is the result of a peptic digestion of the chlorophyll of the larva's food. In a series of his experiments it is also suggested that the red pigment in the epidermis has the same origin.

The origin of segregated pigment in the vertebrates has been much discussed, and it is not yet settled. In the higher invertebrates there is little doubt that dark pigment granules may be elaborated by the epidermal cells as well as by connective-tissue cells. Sections of the mantle epidermis of the mussel, *Mytillus*, for instance, show pigment granules that have quite likely been elaborated by the epidermal cells under normal conditions. Schiedt has shown definitely that dark pigment granules develop under pathological or abnormal conditions within the epidermal cells of the oyster. But even among the Invertebrata the connective-tissue cells are the chief elaborators of pigment granules. These cells assume various shapes and, in some cases, become very highly specialized and are under the control of special nervous impulses. Such is the case with the large pigment cells or chromatophores of the cephalopod mollusks. In the mammals, or at least in man, melanin granules are a connective-tissue product. Ehrmann, in a recent article, advances the following views concerning this conspicuous pigment: "Melanin is intra-cellular, and in the situations where it is present it occurs in the deeper layers of epidermal cells and in certain mesoblastic cells known as *melanoblasts*.[1] The melanoblasts are specialized connective-tissue cells which are round, spindle-shaped, or branching, and are peculiar not only for containing melanin granules, but also for having larger nuclei which stain more faintly than those of ordinary cells. Melanoblasts occur in the upper layers of the corium, are especially noticeable around the blood vessels, and are also present as peculiar structures in the interepithelial lymph-spaces of deeper portions of the epidermis. The substance is a derivative of blood-pigment, the material of which it is formed getting out of the blood vessels into the perivascular tissue spaces, where it is taken up by the melanoblasts and transformed into melanin. The epidermal cells do not elaborate melanin, but absorb it from the melanoblasts in the interepithelial lymphatics."

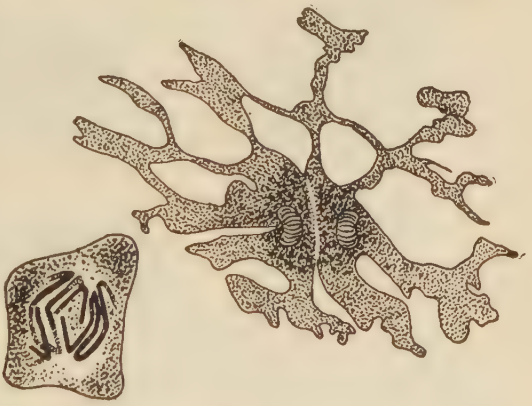

FIG. 234. — Two figures of dividing pigment cells from the skin of a larval salamander. (After ZIMMERMANN.)

The connective-tissue cells that have become highly specialized as

pigment cells divide mitotically. Such mitosis has been observed by Flemming and Zimmermann (Fig. 234).

As an **example of diffused pigment in an animal product** we may take the secretion of the ink-sac of a squid. The ink-sac is a pear-shaped organ lying beneath the integument of the mantle wall. In origin it is an invagination of the walls of the rectum. Its size varies with the size of the animal; its greatest transverse dimension is about one eighth the diameter of the body at the level of the ink-sac. From the epithelium of the fundus of this sac, epithelial pouches are constricted.

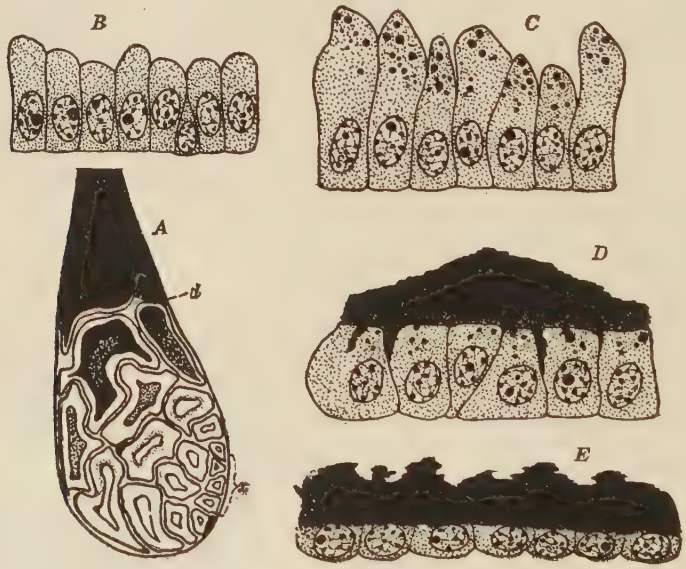

FIG. 235. — *A*, diagram of the arrangement of lobules in the ink-sac of a squid, *Loligo Pealii*. *x*, regions of lobule generation; *d*, regions of discharge of ink from lobules; *B*, epithelium of youngest lobules, no ink apparent; *C*, older epithelium, first appearance of ink in the cytoplasm; *D*, epithelium from still older lobule; much ink formed and stored in lobule; *E*, more ink being produced by cells and ready for discharge. *A*, × 20. *B–E*, × 600.

These, as they are crowded toward the neck of the sac by the continued proliferation of glandular vesicles, increase in size and finally rupture in the vicinity of the neck of the sac (Fig. 235, *A*). The pouch, as it leaves the fundus, is lined with columnar epithelial cells, with oval nuclei. The cytoplasm is homogeneous and free from any pigment (Fig. 235, *B*). As the glandular pouch increases in size, the cells become higher and greater in diameter. At the distal ends of the cells, conspicuous pigmented drops appear (Fig. 235, *C*). As the glandular vesicle continues to grow, the cells become wider, their nuclei become more rounded, while pigment is elaborated at the expense of the cytoplasm. The cells disintegrate distally and thus pour out into the lumen of the vesicle

the pigmented secretion (Fig. 235, *A* and *D*). The glandular pouches eventually burst, and the elaboration of pigment continues at the expense of the cytoplasm until there remains but the nuclei inclosed in a shallow layer of cytoplasm (Fig. 235, *A* and *E*). These nuclei and their cytoplasm finally disintegrate.

In *Vampyrella* we have a beautiful **example of pigmentation which shows how closely diffused pigment may be associated with assimilation.** This simple animal is frequently found living with cultures of fresh-water, green Algæ. It consists of a film of colorless protoplasm bearing numerous refractive granules. The sheet of protoplasm, when containing no food bodies, is little more than a micron thick. When

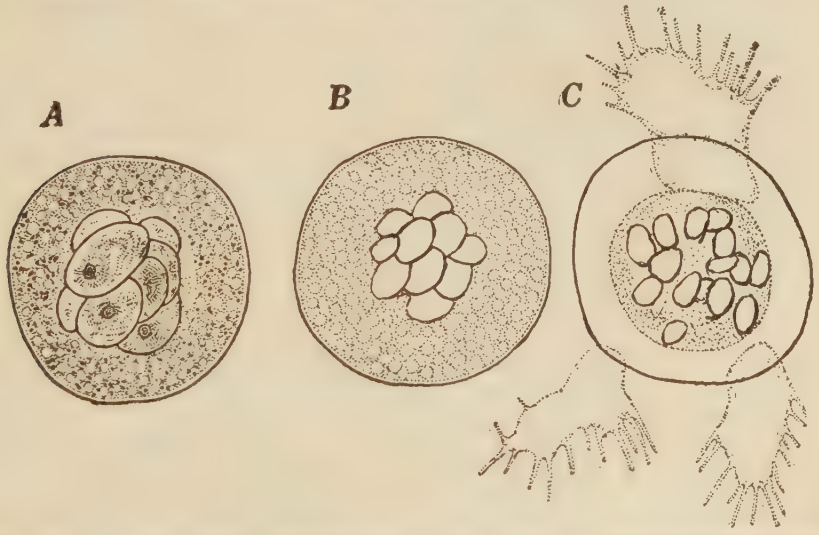

FIG. 236. — *A–C*, three stages in the life-history of a *Vampyrella*. *A*, with freshly ingested green food plants; *B*, food plants turned to a reddish brown; *C*, the animal departing from its encysted state as three reddish brown individuals. × 725.

food is ingested, the protoplasm forms a rounded mass about which an encysting membrane is formed. The animal remains quiet while the food is being digested. As digestion proceeds, the plants taken in as food change from green to brownish red. The food decreases in size as its digested parts are absorbed from the food vacuole. The protoplasm becomes pigmented by the assimilated food, so that when the protoplasm breaks from the cyst as a free animal or animals, it is conspicuously colored brownish red (Fig. 236, *A*, *B*, and *C*).

Metopus furnishes an **example of segregated pigment within a cell.** *Metopus* is a simple, ciliated infusorian found very frequently in old infusions. It will be readily recognized from our Figure 237. At the anterior end is a patch colored dark brown to black. This,

when magnified five hundred diameters or more, is easily resolved as a group of oval to rounded pigment granules (Fig. 237, *pg.*).

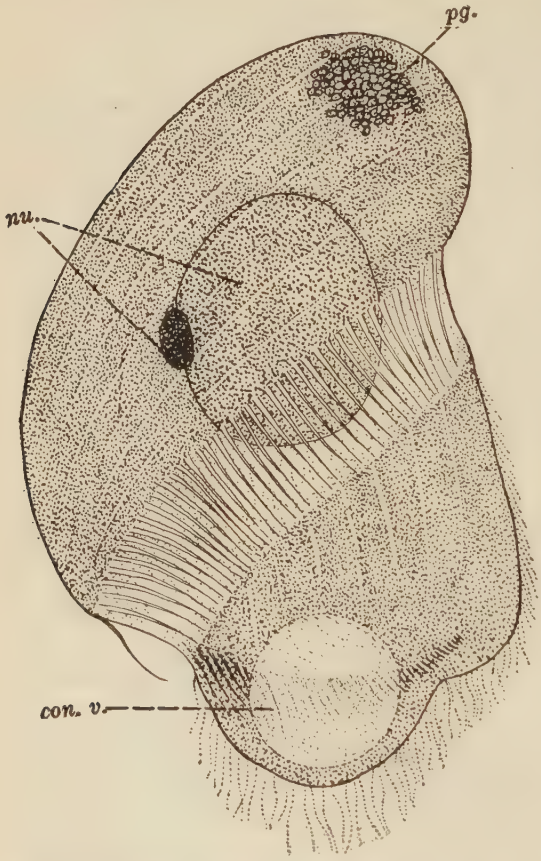

FIG. 237. — Individual of the protozoön *Metopus*. *pg.*, segregated pigment granules; *nu*, nuclei; *con.v.*, contractile vacuole. × 1000.

The cells covering the surface of the **mantle of a mollusk**, like *Mytillus*, are columnar, with oval nuclei near their base. The distal ends are naked, but their cytoplasmic structure is hidden by numerous pigment granules. These granules become less numerous near the nuclei and are wanting at the bases of the cells (Fig. 238).

Connective-tissue cells are the most common elaborators of pigment. In many cases they become specially modified as pigment-bearing cells. Such cells are found in the skin and peritoneum of the fish *Ammodytes*. These pigment cells are stellate with wedge-shaped rays. The one or two conspicuous nuclei lie within the central cell mass. It is within this centrally disposed cytoplasm that the pigment granules are most numerous (Fig. 239). This is an example of what is known as a pigment cell.

In the last three examples the pigment was mostly confined to a particular region of the cell. In certain hepatic cells this is not the case. The **hepatic cells of** *Cryptobranchus* are polygonal with a well-rounded nucleus inclosed in a cytoplasmic layer. The cytoplasm, when not highly pigmented, contains many vacuoles. Pigment granules are elaborated more or less uniformly throughout the cytoplasm (Fig. 240, *B*). This pigmentation may be so great as to conceal all cytoplasmic structure (Fig. 240, *C*).

The chromatophore of the squid is a complex structure composed of a central pigment cell surrounded by smaller cells, which send their slender bodies in a radial fashion from the pigment cell to supporting connecting tissue. These cells are contractile and respond to nervous impulse brought by a special nerve supply. In this way the shape of the chromatophore is modified. Change of form alters the color of the chromatophore. Chun has shown that this complex chromatophore arises from a single embryonic connective-tissue element, which is an oval cell as represented in Figure 241, *A*.

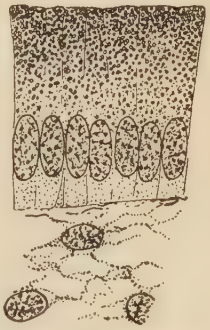

FIG. 238. — Pigmented epithelial cells from the external covering of the mantle-fold edge of a mussel, *Mytillus*. × 1500.

This cell is at first small and contains a single nucleus. The nucleus is eccentric, being placed at the extreme end of the cell. This is apparently so, because of the presence of an astral figure which probably represents a centrosome with its rays.

As the cell grows, it may be noticed (Fig. 241, *B*) that the peripheral cytoplasm or ectosarc becomes differentiated from the remainder of the cell by becoming clearer. It also sends out sharp-pointed processes, which are well seen in (*C*). These processes increase much in length and thickness, and when the cell is much larger, some of them, usually three or more on one side, form a contact with some nerve fibers which come from a nerve plexus in the integument. This condition is shown in Figure 242.

FIG. 239. — Pigment cell from the peritoneum of the sand-launce *Ammodytes*. Fully extended. Arms can be retracted until the cell is irregularly round. Two nuclei, between which the non-pigmented centrosphere appears. × 180.

The next feature to develop is a differentiation of the structure to enable the cell to perform its dual function. These two functions are: the production and maintenance of a pigmented area, and the mechanical operation of that area by a muscular apparatus. A multiplication of the nucleus takes place; in Figure 242 the single nucleus has already divided into two. In Figure 243 there are four nuclei, and two of them

have become modified. They are found, here, in the central area of the cell which is now a syncytium, and this area, which was already differentiated from the ectosarc, has become further separated by the formation of a distinct sac about its content of pigment-bearing cytoplasm. The sac-wall is but partly formed in Figure 242.

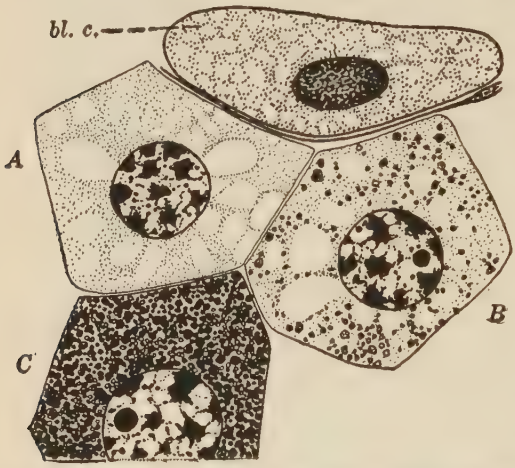

FIG. 240. — Three liver cells from the salamander *Cryptobranchus*. They show, in *A*, *B*, and *C*, three successive stages of pigmentation. *bl.c.*, blood cell.

The young, but completed, *chromatophore* is seen in Figure 244. Marked changes have taken place. The pigment sac is inclosed by a tough, membranous wall. But one nucleus remains within the sac, and this is larger and is specialized to direct the pigment-forming activities of the inclosed cytoplasm. The other nuclei have multiplied by amitosis and have migrated outside the sac, so that one lies in the basal portion of each cell process. These nuclei control the myo-fibril-forming activities of the cytoplasm in the processes whereby they render them contractile. The processes are now able to stretch the central pigment sac out until it becomes very broad and visible. And relaxing, they allow it to contract by its elasticity and become almost invisible. The chromatophores are arranged in two or more different sets, and all the members of each set are connected with a common nerve plexus. At the same time the different sets are independent of each other as to nervous control, and so but one or two or all or none of them may be expanded at a time. As each set contains a differently colored pigment in its pigment sacs, we have an explanation of the rapid changes of color which pass over a squid or octopus at different times. At one time it may be red, the next transparent, and presently turn

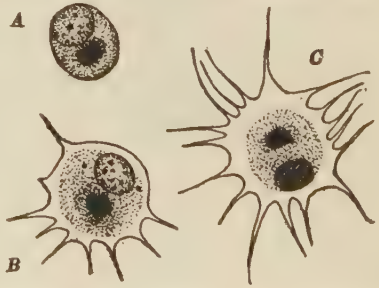

FIG. 241. — *A*, *B*, and *C*. Three youngest stages in the development of a cephalopod's chromatophore. Each stage shows a nucleus and centrosphere. The two older ones have put out processes. (After C. CHUN.)

brown or yellow. Many other animals, as frogs and lizards, change their color in much the same way.

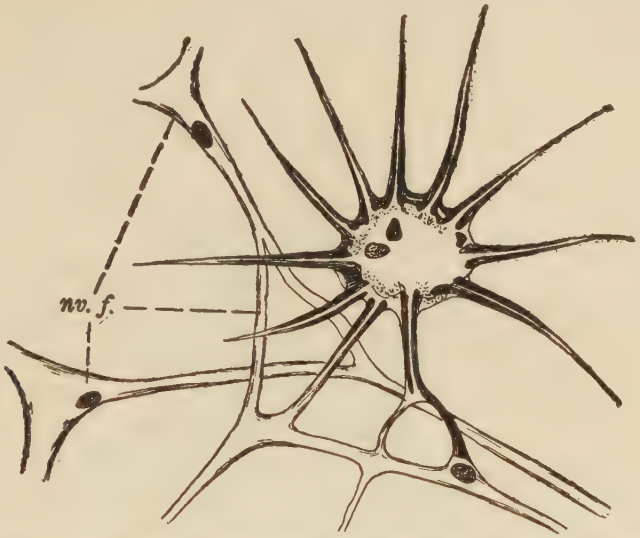

FIG. 242. — An older form of the chromatophore, with two nuclei and well-developed processes, two of which have established connections with nerve fibers, *nv. f.* (After C. CHUN.)

Technic. — The only special technic to be mentioned in this place is the methods used to depigment the various sorts of pigmented tissue. This process is necessary in order that the structure and relations of the pigment cells themselves may be determined. It is sometimes needless owing to a small amount of pigment or to a light color and transparency of this material. It is always a supplementary proceeding, and should never be used without a proper relation to the study of the unchanged tissue. When much pigment is present, the tissue should be fixed and hardened first and

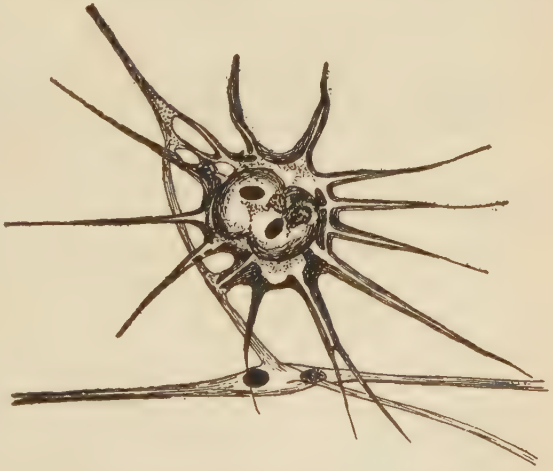

FIG. 243. — Still older chromatophore of a cephalopod. The nuclei are four in number and have become differentiated. The central, pigment-containing region is beginning to appear. (After C. CHUN.)

then steeped in water containing 3 per cent each of nitric and hydrochloric acid. Other mixtures may be used, as chloroform containing a drop or two of strong nitric acid. Or 50 per cent alcohol warmed and saturated with picric acid will depigment. Alcohol, glycerin, and

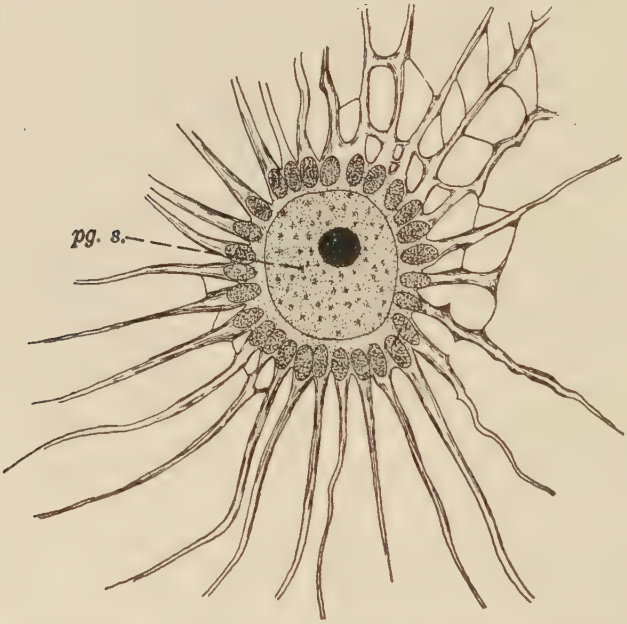

FIG. 244. — Well-developed chromatophore of the same cephalopod. Processes completed and extensive connection with a nerve plexus. Nucleus in base of each process. *pg.s.*, pigment sac containing pigment granules and one nucleus. (After C. CHUN.)

water in equal parts, through which chlorine gas is passing, is also a good reagent.

The fixation will itself often remove the pigment. This is not to be recommended, as it is usually associated with a poor fixation. Well-fixed tissues nearly always show the pigment in good condition.

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CHAPTER XV

STRUCTURES OF ALIMENTATION

POTENTIAL energy is acquired by animals in two ways—(*a*) as oxygen taken unchanged from the atmosphere, and (*b*) as matter taken as food and transformed. The acquiring of energy and the accumulation of material for growth through the transformation of food materials is what we call alimentation. This process takes place in every living animal. The process is a double one, involving the dissolving or digesting of the food and the absorbing of the food after it is digested. The structures concerned with alimentation must, therefore, provide cavities for the retention of the food while it is being digested, and surfaces which can absorb the food when once digested. *Food vacuoles* and food cavities retain the food until digested. The surfaces of such vacuoles and cavities discharge the necessary digestive juices and afterward absorb the digested food. In the Protozoa, where an individual consists of but one or a few cells, there can be no inter-cellular food cavity or enteron; hence digestion must take place *within* a cell in food vacuoles. These unicellular animals secure their food by means of protoplasmic processes, called pseudopods, flagella, cilia, and membranelles.

By these structural devices food, suspended in some water, is carried within the cell where the cytoplasm surrounds it on all sides, forming a rounded, digestive vacuole. This vacuole is not a permanent structure of the cell.

The size of the vacuole is not constant. Its place of origin may be no fixed region of the cell, as in *Amœba*, *Vampyrella*, and other naked Rhizopoda; or the food vacuole may arise at a definite region of the cell, as is the case in the Infusoria of which *Paramœcium* is a representative; nor is the position of the food vacuole once formed stationary; it is carried with the cyclosis of the cell. The vacuole receives the digesting fluids secreted by the cell, and retains the food until digested. Its surface absorbs the digested food. At the completion of this double process the vacuole moves to the surface of the cell to discharge the waste matter brought in with the food and to disappear as a feature

of the cell. This is the fundamental, structural device by which all intra-cellular alimentation is accomplished.

In all Metazoa the alimentary tissue is differentiated and distinct from the other tissues of the body; and in all cases it is an epithelial tissue. Despite the presence of a distinct alimentary tissue, however, and until a definite food cavity or enteron is established, pseudopods, flagella, cilia, and food vacuoles are the *only* structural, alimentary features. The endoderm of the Porifera or sponges is an alimentary tissue composed of a simple collared epithelium, each cell of which is provided with a flagellum. This epithelium lines a cavity which is not an efficient place for digesting fluids to act upon food, because of the currents of water that constantly stream in and out of it, and which would carry away the secretions. Food, therefore, is thrown into the cell body of an endodermal cell by means of the flagellum, and alimentation takes place within a food vacuole in a manner analogous to the process used by the Infusorian.

The food vacuole limits the size of the particles of food consumed and is, therefore, not an highly efficient alimentary structure. With the progressive development of the food cavity or enteron, the food vacuole becomes less frequent.

In the Cœlenterata, polyps, and jellyfish, a food cavity or enteron is formed which is open at but one region, and thus supplies a place for digestive fluids to act upon food outside of the cell body. Here pseudopods and cilia attend both food vacuoles and an enteron. Besides the cells, bearing food vacuoles, there are others which elaborate digestive fluids to be discharged upon the food and to digest it in the enteron.

Before the enteron becomes an open tube, it is supplied with a muscular coat which, by a churning action, aids the extra-cellular alimentation. In some of the platyhelminthes the endodermal cells may bear food vacuoles. The simple columnar epithelium, however, is an alimentary tissue acting chiefly upon food contained within the lumen of the enteron. Here there is a slightly evident differentiation between cells of digestion and cells of absorption. But the cells so differentiated are not assembled to form two tissues.

In the Annelida and all higher forms, the enteron is made more efficient by a stomodæum and a proctodæum. With this advance in the formation of the general food cavity, there arises a differentiation of alimentary tissues into *digestive* and *absorptive* tissues.

This differentiation together with the development of a separate internal digestive cavity enables the organism to use a great variety of foods, some of which are bulky in nature or hard or tough. Such food must be first mechanically cut off to be swallowed, and some of it ground

up to become a suitable object for the digestive juices to act upon. It may also be crushed or ground after being partly digested. This work is called *mastication.* It must then be acted upon by the tissues differentiated to prepare it for absorption. This process we shall designate as *digestion;* when digested the food is ready for other tissues whose surfaces will absorb it. This is *absorption.* All of these processes, except perhaps mastication, are differentiations of the simple alimentary processes that take place in the Protozoa and in the alimentary epithelium of the sponges and to be a degree in some higher forms.

All masticating structures may be considered as mechanical, digestive structures. In all forms where an enteron is present, the muscular layer of the alimentary tube must be considered to be a masticating tissue as well as a structure for moving the food through the alimentary canal. As it becomes more highly developed, cilia become less frequent. In the Cœlenterata the muscular wall of the enteron is composed of the layer of striped muscle given off by the ectodermal cells. In all these forms it is of very low development and probably secondarily concerned with alimentation. In the platyhelminthes it has become a definite layer of non-striped muscle fibers closely applied to the basement membrane of the endodermal epithelium. It is here clearly concerned primarily with alimentation. This structural feature persists throughout all higher forms. The muscular layer of the alimentary tube becomes intensified in various regions to act as specialized masticating structures. The "gizzards" of annelids and vertebrates, and the stomachs of mammals and some crustaceans all present examples of such muscular development. The epithelium of these regions becomes more compact to serve as protective rather than secreting or absorbing structures.

In certain fishes the action of the muscular wall of the stomach is aided by calcareous structures laid down by the epithelium of the enteron. The most common and most highly specialized masticating structures arise from the surface of the stomodæum. The honey-stomach of the bee and stomach of the lobster are the dilated posterior parts of the stomodæum that have developed chitinous masticating structures which operate together with the special muscular supply of this region. The radula of a snail is a cuticle of chitin formed by the epithelium of the mouth and œsophagus. The teeth, most common of all masticating structures, are but highly specialized integumentary structures. The transition from a scale to a tooth is easily seen in the region of the mouth and on the jaw of an elasmobranch.

Certain glands constitute a second class of accessory alimentary tissues. These are more or less complex in their structure and may arise from any region of the alimentary canal, but they always open into the lumen of this canal. Such accessory structures vary with the character

of the food eaten. The calcium carbonate glands of the earthworm are *œsophageal glands* which have arisen, as is held by some investigators, to supply an alkali to neutralize the great amount of acid taken in with the food. Many of the so-called salivary glands of vertebrates have been differentiated as structures to supply fluids that mechanically aid digestion by furnishing a fluid for softening and lubricating the food. These glands are known by the character of the secretions given off as mucous glands. The mucous glands are represented by the palatine, the doudenal (Brunner's) glands, and the glands of the large intestine. These glands vary in complexity, but all elaborate a thick mucus, which serves primarily to lubricate the food on its passage into and out of the alimentary canal.

The regions of the alimentary tube, which are little more than passageways for food materials, form another class of accessory tissues. These regions are always tubular, with lumina that are narrow when not functioning and with highly developed walls. The muscular coats are thickened and supported by an intensified connective tissue. The blood supply in these regions is less than in the more active digestive regions of the alimentary tube. The epithelium of a conducting region is always heavy and suited to withstand abrasion. In some cases a heavy cuticle of chitin or other dense material is formed. When this protecting cuticle is developed, the epithelium is simple and columnar. Resistance to abrasion in the absence of a cuticle is met by stratification of the lining cells. In most cases there are frequent strata of the resting cells. In many cases certain digestive and accessory glands pour digestive and lubricating fluids into these conducting tubules. The pharynx and œsophagus are examples of such structures.

Each cell of an absorbing tissue must come into actual contact with the food, and consequently we find these epithelia in the central cavity of the digestive tract to which the food is confined; on the other hand, as long as the digestive tissues have a canal leading from them to the lumen of the alimentary tube, they may retreat as glands to remote and various regions of the body. Digested foods vary much less than foods not digested. Because of these two facts we find absorbing cells and tissues much less differentiated than digestive cells and tissues.

Absorbing cells are not well defined. Indeed, it may be said that all cells coming in contact with digested food may to a certain degree be absorptive, despite any peculiar structure they may have. The most efficient absorbing tissue is one that presents the greatest number of living cells in contact with digested food. In regions where we have stratified epithelium, therefore, we find the least efficient absorptive tissues. A characteristic absorbing epithelium is always a simple columnar one; such a tissue stands exposed to the lumen of the enteron;

its extension is increased by folds and villi and other evaginations arising from the inner surface of the enteron. The cells lining the posterior half and more of the alimentary tube of invertebrates and those found upon the villi and folds of the intestine of vertebrates furnish good examples of absorptive cells constituting an *absorptive tissue.*

The essentially digestive tissues are mostly glandular. We meet with such diversity among them that we find ourselves at a loss for a morphological basis by which to classify them. As all structures exist purely for the purpose of performing certain functions in some particular manner, we may be justified in adopting a physiological basis for the classification of these tissues. The physiological study of the digestive tissues of invertebrates does not as yet afford a basis for an extensive classification of these tissues. All glands belonging to the alimentary tube and the anterior third or less of an alimentary tube may be considered as digestive tissues.

Among the vetebrates the digestive tissues may be classified for our purpose as: *pancreatic*, *gastric*, *serous*, and *hepatic.* This physiological basis holds only for the higher vertebrates, where these functions have been assigned to particular tissues. If the experiments of physiologists are to be considered as final, the cells of the villus region of the midgut folds of insects are both hepatic and pancreatic, and the hepatic cells of mollusks are also pancreatic. The digestive cœca of the starfish and *Amphioxus* have been shown physiologically to be pancreatic, though it is quite probable that they have other functions as well. By these conclusions we may appreciate the fact that among the lower animals where digestive tissues have been differentiated from absorbing tissues, there has not yet arisen a differentiation of the digestive tissues into pancreatic and gastric tissues. The tissues of the gastric cœca of the invertebrates are spoken of as *hepato pancreatic* tissues. In the higher forms, where this fourfold specialization has been effected, most digestive tissues are glandular.

The *pancreatic tissues* are those that secrete ferments that are active in an alkaline medium. These ferments are *ptyalin*, *trypsin*, and *steapsin.* These are the most active digestive tissues. The pancreatic epithelium of mammals resembles somewhat a serous epithelium. These tissues are assembled to form the vertebrate organ called a *pancreas.*

The *gastric tissues* elaborate a ferment that is active in an acid medium. This ferment is pepsin. These tissues in mammals are composed of two kinds of cells: the ordinary gastric cells that elaborate the ferment, and the acid cells which supply the acid necessary for the action of the ferment. The alimentary tissue found in the gastric glands of a mammalian stomach shows these two kinds of cells. In a bird they are found in separate tissues.

The *serous tissues* of digestion elaborate a watery secretion that contains the ferment ptyalin or some other digestive ferment. In secreting ptyalin they resemble pancreatic tissues. The serous tissues functionally represent grouped albumen cells or serocytes, which are so frequently encountered isolated among the alimentary tissues of invertebrates. The parotid gland and certain lingual glands at the base of the tongue are pure serous glands. The submaxillary gland presents both mucous and serous cells; hence it is called a *mixed gland.*

The hepatic tissues secrete bile, which is a fluid active in the digestion of fats. This tissue also has the power to elaborate glycogen from certain soluble carbohydrates. With this function the hepatic tissue becomes a storehouse of energy. In the lower forms other tissues than the gland known as the liver may have this accessory function, as the so-called liver cells found in the foot and dorsal mantle region of the

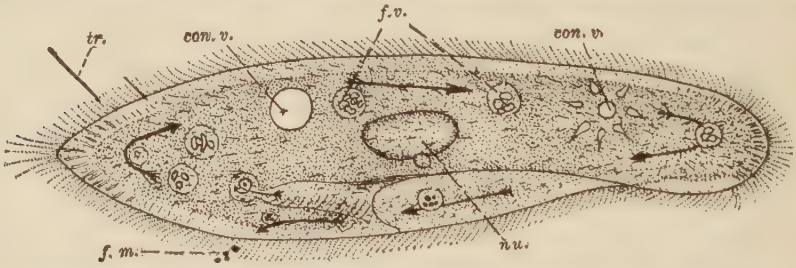

FIG. 245. — Individual of *Paramœcium caudatum.* Arrows show course of food vacuoles (*f.v.*). *nu.*, nuclei; *con.v.*, contracting vacuoles, one empty and one full; *f.m.*, fecal matter; *tr.*, discharged trichocyst. × 375.

fresh-water mussel. All vertebrates have hepatic glands that elaborate glycogen.

Certain alimentary tissues have been differentiated as structures no longer directly concerned with alimentation, as for example the poison glands of certain reptiles. These will be considered under another heading.

Examples of intra-cellular alimentary structures. — *Paramœcium* is a very common protozoön that is found in most infusions. It is a slipper-shaped creature with a rounded, narrow anterior extremity and pointed at the posterior end. Extending from the anterior to the middle of the body there is a lateral oral groove, which leads in a slight spiral manner to the gullet at its posterior end. By means of cilia currents of water are created, which bear food along the oral groove into the gullet. At the base of the gullet the food and water taken in with it form a spherical food vacuole. The vacuole becomes too large to withstand the impact of the water entering it from the gullet and breaks away. It is then slowly carried along with the cyclosis of

the cytoplasm. Into this vacuole the cytoplasm empties digesting fluids, which act upon the food. The digested food is absorbed by the surface of the vacuole, and the non-digestible parts of the food are thrown out of the cytoplasm at a definite part of the surface of the cell. When the vacuole is thus emptied, it disappears (Fig. 245).

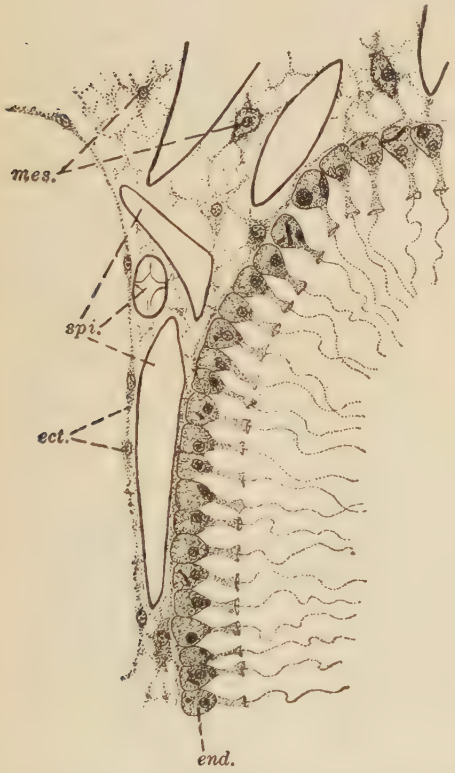

FIG. 246. — Part of the body of a sponge, *Grantia*. *mes.*, mesoderm; *ect.*, ectoderm; *end.*, endoderm; *spi.*, spicules. The black objects in the round bases of some endodermal cells are food particles.

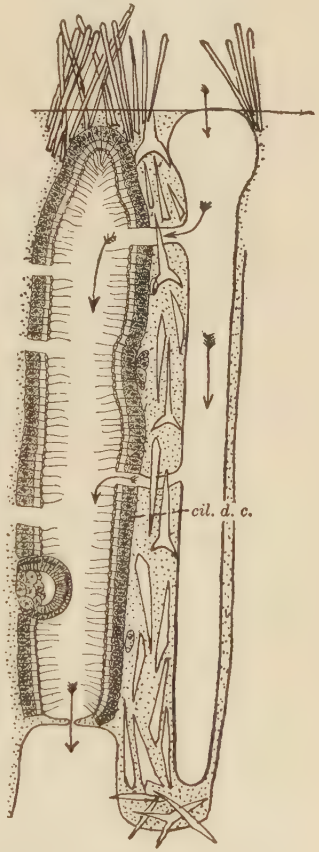

FIG. 247. — A sectional diagram of a water canal of the sponge *Sycon gelatinosum*. *cil.d.c.*, ciliated digestive cells. (After PARKER and HASWELL.)

In the sponges there is a differentiation of the cells into ectodermal, endodermal, and mesodermal tissues. The endodermal cells line the internal cavities and form the alimentary tissue of the sponge. These cells are collared cells, each bearing a flagellum (Fig. 246). The water, bearing food, is brought into the endodermal canals at certain places and driven out at other places by the actions of the flagella (Fig. 247).

FIG. 248. — Several digestive cells from the endoderm of *Hydra*. *sec.g.*, secretion granules; *f.v.*, food vacuoles; *nu.*, nucleus. × 870. (From a preparation by A. H. TUTTLE.)

In this way the currents of water continually sweep by the alimentary tissue, preventing digestive secretions from acting upon food that may lodge outside of the alimentary cells. The food must therefore be taken into the cell bodies to be digested within food vacuoles; comparatively large bodies are taken into these food vacuoles (see Fig. 246).

An example of simple intra-cellular alimentary tissue. — In the Cœlenterata and Platyhelminthes the endoderm has been differentiated to perform the function of alimentation. It is of interest here to note that the alimentary tissue lines a cavity which is open at but one end. This affords a place for food to lodge, protected from any sweeping currents of water. Here we meet with, in our study, the first cavity that is to any degree efficient as an extra-cellular cavity or enteron. The cells lining this cavity are all of tall columnar forms. The oval nuclei lie near the base or in the lower third of all the cells. The distal ends of the cells are expanded and may bear many vacuoles. There is here an interesting differentiation of the alimentary cells into two types of cells: the ordinary cells which we may call the *absorptive* cells, and the albumen cells which elaborate a digestive ferment; hence we call them the *digestive* cells (Figs. 248 and 249). The cytoplasm of the albumen or digestive cells is much denser than that of the absorbing cells. They are usually shorter than the absorbing cells. Their secretion products are elaborated in the form of spherical bodies at the proximal end. The nuclei are easily distinguished from those of the connective-tissue cells, but the nuclei of digestive and absorbing cells cannot be distinguished. It is interesting to note that both in *Hydra* and *Bdel-*

FIG. 249. — Six digestive cells from the enteron of *Bdellura candida*. They show some food vacuoles and secretion. In both this and the preceding example the dark cell probably secretes a different ferment from that produced by the lighter cells. × 870.

lura the digestive cells are most numerous in the region of the opening of the enteron. At no place, however, are these digestive cells assembled to form a *tissue*. Food vacuoles yet function to a certain degree. In certain absorbing cells such vacuoles are occasionally found (see Fig. 248).

Examples of Accessory Digestive Structures

Masticating Structures. Gizzard. — **The gizzard of an earthworm** is a region of the alimentary canal in which the muscular layer is most highly developed. The layer of circular muscles lies next to the submucosa, and is much thicker than the outer longitudinal layer. The elements of this muscular tissue are smooth, non-striated muscle cells (see Fig. 96). The epithelium is composed of columnar cells. The cytoplasm of these cells is finely granular and homogeneous. The oval nuclei lie at the middle of the cell. The basement membrane is clearly defined. At their distal ends the cells elaborate a heavy cuticle which is constantly being formed as it is worn down by abrasion in grinding the food. Many lymphocytes find their way through the basement membrane into the epithelium (Fig. 250).

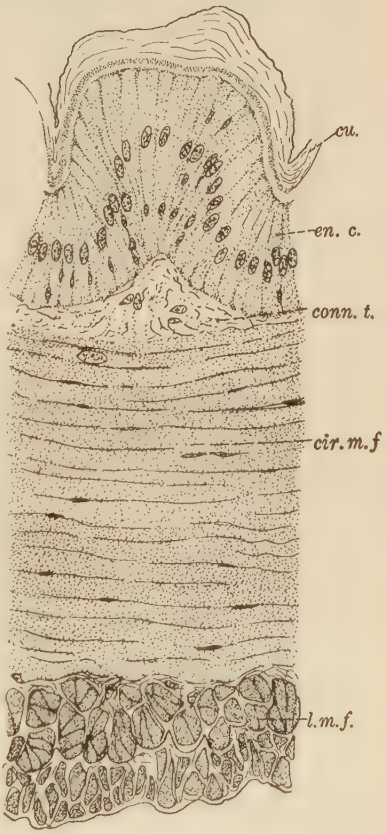

Fig. 250. — Transverse section of part of the gizzard wall of an earthworm, *Lumbricus*. *cu.*, cuticle; *en.c.*, endoderm cells; *cir.m.f.*, circular muscle fibers; *l.m.f.*, longitudinal muscle fibers; *conn.t.*, connective tissue. × 400.

In **the gizzard of vertebrates, as represented by the bird's gizzard,** we find that a short portion of the digestive tube is enlarged and provided with unusually thick muscular walls in order that there may be grinding power to triturate the food. This is what has taken place in the worm's gizzard, and the similarity is further made apparent by the fact that a heavy cuticular layer of substance is placed on the internal surface to protect the soft

parts themselves from the grinding action of the stones that are kept in the lumen to reduce the food material.

As in the worm's gizzard, the cuticle is formed by the inner layer of simple epithelium against which it lies (Fig. 251). There is the difference here, however, that the epithelial layer of the bird does not secrete the lining directly from its primary surface as a solid layer but from a close-set layer of simple, tubular glands into which it is thrown. The protective material is produced from these glands and issues from their mouths as a series of liquid or semiliquid strings, which spread out and fuse with one another to form the cuticle. They are speedily hardened superficially by the acid that comes down with the food from the proventriculus, and as fast as the inner, wearing surface is ground away it is replaced from the other surface by the glands beneath.

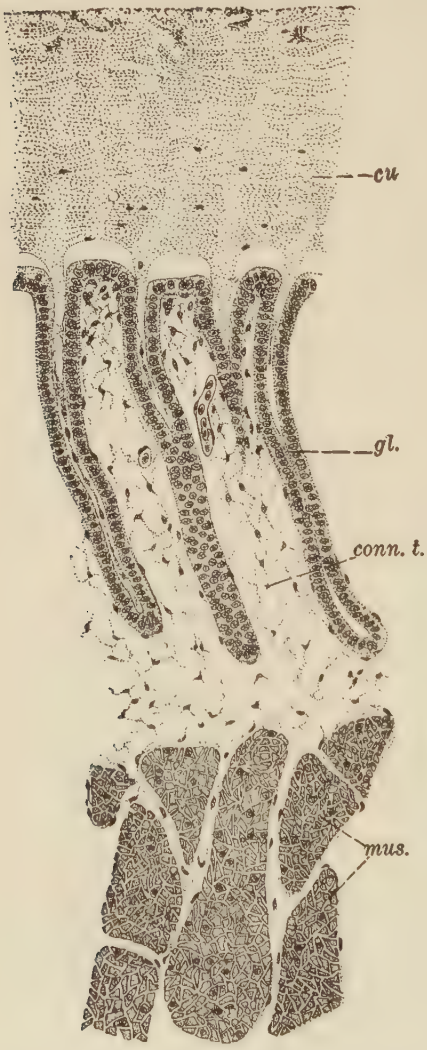

FIG. 251. — Part of a section of the wall of the gizzard in an English sparrow. *cu.*, cuticular protective layer; *gl.*, simple tubular glands in which the cuticular layer is secreted as a fluid; *conn.t.*, connective tissue; *mus.*, muscle. × 750.

These glands dip into the submucosa and form a considerable layer. Their secreting cells are said to produce no digestive juice, although the cuticular lining is thought to contain such materials and is used as a cure for some forms of indigestion.

Among the many very peculiar masticating structures found in animals are some of the "gizzards" that may be seen in the anterior part of the digestive tract of fishes. **One such gizzard will be described in the harvest fish, *Seserinus paru.***

This organ consists, unlike the other gizzards, of an enlarged, muscular portion of the œsophagus, whose inner surface is beset with tooth-like structures and invaginated between these teeth into the glands. The epithelium is primarily a stratified one and continues to possess this characteristic where it is involved in the tooth formation. In its relation to the glands it is carried down into them, its more distal cells showing a marked secretory activity, probably of mucin. In the fundus the outer cells have become so markedly columnar and so active as secretory cells that one has difficulty in deciding that it is not a true, simple, columnar epithelium.

The presence of a certain amount of stratification at the base, however, together with its origin, furnish ample evidence that it is in reality a pseudo-stratified form.

The teeth are mesodermal in formation, the epithelium taking no share in their formation. They arise from a common base which is a stiff basket work of hard tough fibers that arise in the subepithelial connective tissue through the activities of some of its cells. The shell thus formed is elastic enough to allow of the grinding movements of the gizzard. In fact, it is not complete in the median line, thus forming two halves which grind upon one another.

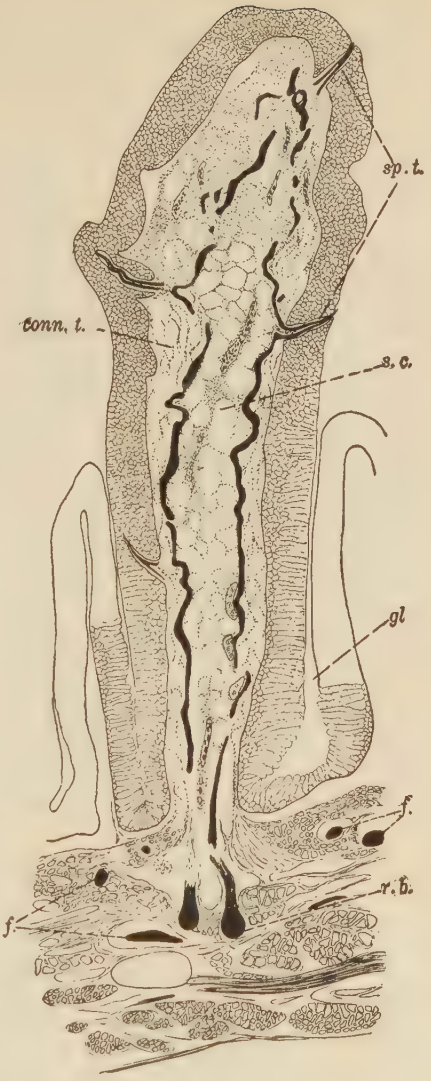

FIG. 252.—A single compound tooth from the œsophageal gizzard of the harvest fish, *Seserinus paru*. *f.*, transverse and oblique section of the deep and hard reticulum, from which the tooth arises; *r.b.*, two sections of the basal ring, from which the sides of the skeletal core (*s.c.*) arise to support the papilla; *sp.t.*, spike-shaped cusps which pass through the stratified epithelium; *conn.t.*, connective tissue between skeletal core and epithelium; *s.c.*, soft core of connective tissue with fat cells, blood vessels, and lymphatics; *gl.*, glands at base of tooth. × 80.

Each tooth (Fig. 252) arises from this basis of which it is a part and projects into the stratified epithelial papilla, which it conforms to in shape, but does not quite fill. It is a hollow shell of basket work, irregularly reticular, so that but few fenestræ can be seen in its sides. It is narrow at the base, with thick sides that arise parallel and diverge as they pass distally until they arch over and meet at the top. This top is thus broad and blunt. The whole structure has much the shape of a balloon or, still more, that of an ordinary incandescent electric-light bulb. Its walls remain at a little distance from the stratified epithelium, being kept separate by a layer of lax connective tissue. The inside of the tooth is filled with a connective tissue that contains much lymphatic tissue in its meshes as well as an abundant blood supply.

As so far described the structure could not act as a tooth at all, being covered by a thick, stratified epithelium. It possesses, however, on its upper, outer surface a series of strong, hollow spines which project through the stratified epithelium and come in contact with the food. At the point where they pierce the epithelium the basement membrane is reflected distally along the spines, and the epithelium sends a close-fitting layer a short distance proximally around the spike. Thus a *gum* is formed. The interior of the spike is filled with very active cells, which line its interior and through whose agency its walls are formed and maintained.

This tooth is not used to grind with, but its sharp spines, reaching a short distance into the lumen of the digestive tube, tear and grate the flesh of the animals which are eaten by the harvest fish, and thus prepare them for digestion. In some other fishes there is a gizzard with a similar structure, except that the teeth, instead of lying inside the gum with only the tips of their spikes projecting through its surface, stand bodily out from the gum and are hard and thick with a white, polished surface. They have shorter, thicker spikes and are used for direct mastication.

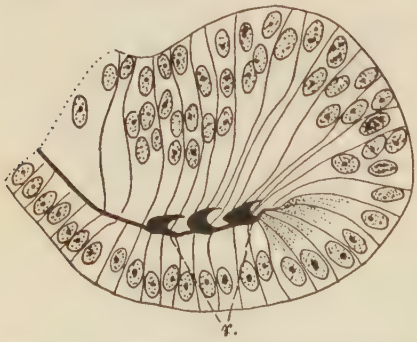

FIG. 253. — Epithelial fold in which the *radula* or toothed tongue of *Helex pomatia* is developed. *r.*, teeth of radula. × 350. (After SCHNABEL.)

The radula of the snail, *Helix*, is composed of numerous chitinous processes which are directed posteriorly. These are produced in a certain epithelial fold of the mouth by a particular set of epithelial cells (Fig. 253). They may be regarded as parts of the cuticle which these cells form. As they are formed they are pushed anteri-

orly over other columnar cells to be exposed on the radular surface where they function as cutting and masticating structures.

The honey-sac of a bee is the dilated posterior end of the stomadœum. The wall of the sac has a muscular coat. It is lined with a low columnar epithelium which secretes a dense cuticle. At the posterior end the sac is thrown into a rounded prominence which projects anteriorly into the lumen of the sac. The cuticle over the curved surface is modified to form numerous pointed processes, which are directed towards the opening at the apex of the prominence. This opening leads through a narrow passage into the intestine. The wall of this passage is highly muscular. Its lumen has many long, cuticular setæ which are directed posteriorly (Fig. 254). These two sets of cuticular processes function as mechanical aids to digestion. By means of them nectar or pollen is passed into the intestine as demanded. When pollen is demanded, the short processes, by their action, carry pollen grains with a certain amount of nectar into the narrow passage leading to the intestine. As this is being done the passage is closed posteriorly. A constriction then passes anteriorly, according to Cheshire, sending the nectar back into the honey-sac and leaving the pollen grains held by the long, cuticular setæ. These then pass the pollen into the intestine with little or no nectar.

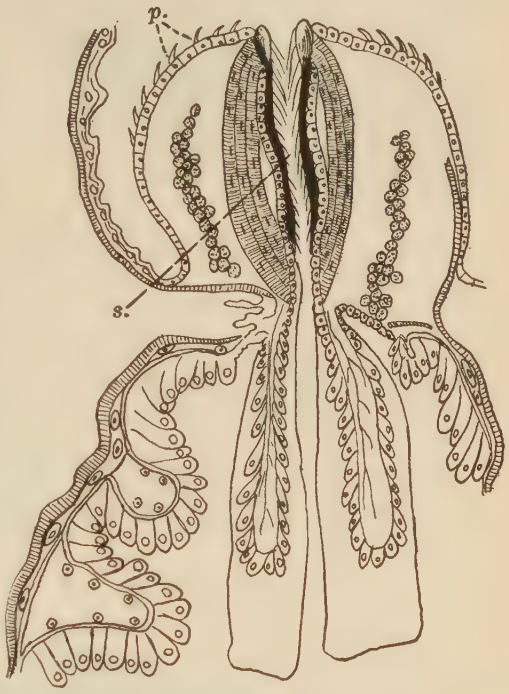

FIG. 254. — Longitudinal section of honey-sac of bee. *p.*, pointed processes on curved epithelial surface; *s.*, setæ on inner lining of muscular passage. (From PACKARD after CHESHIRE.)

Teeth. — The structure of a tooth is perhaps best studied in a series of developing teeth. We have chosen **the teeth of a dogfish** for this study. In the embryo dogfish the mouth is lined with a stratified epithelium, the basal cells of which tend to be columnar. Along the inner margin of the jaw this stratified epithelium forms a crescent-shaped

groove. The anterior wall of this groove becomes a primitive *dental ridge*. Over the surface of this ridge structures arise which are fundamentally similar to embryonic integumentary scales. At the fundus of this dental groove these structures are continually forming, and travel anteriorly by a movement of the whole epithelium towards the mouth of the groove. In its earliest stage the tooth appears as a papilla formed of a core of mesenchyme and an epidermal sheath (Fig. 255). The columnar cells forming the lower stratum of the epidermis enlarge to become the enamel-forming tissue. The other layers of the epidermis disintegrate. In Figure 256 remains of epidermal (*B*, *e.p.*) cells are lying over the enamel cells. The cells of this enamel tissue become much taller; their nuclei move towards the distal ends of the cells, and the cytoplasm becomes highly vacuolated at the proximal ends of

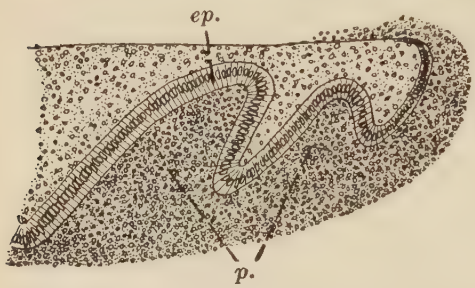

FIG. 255. — The two earliest stages of tooth-formation in the fundus of the dental groove of a young dogfish, *Acanthias vulgaris*. *p.*, mesodermal papillæ; *ep.*, basal layer of the stratified epithelium. × 80.

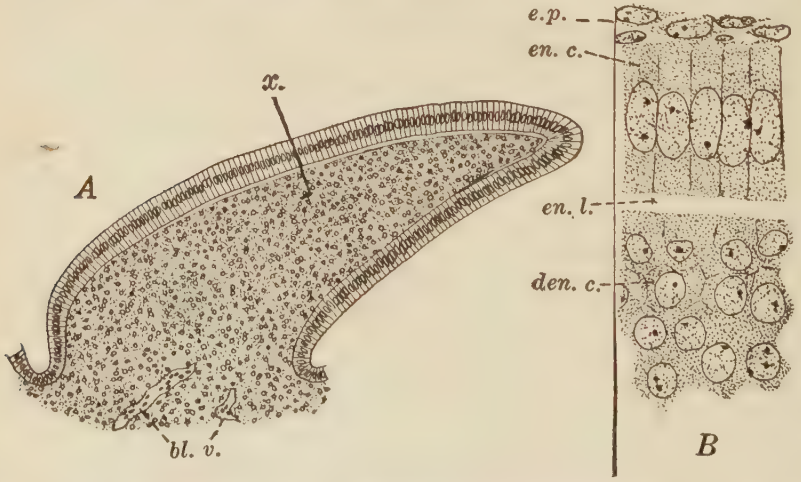

FIG. 256. — *A*, larger developing tooth of *Acanthias* than that shown in Figure 255. *bl.v.*, blood vessels beginning to enter the papilla. Longitudinal, vertical section × 80. *B*, enlarged detail of *A* at *x* to show the enamel cells (*en.c.*) and the young layer of enamel (*en.l.*); *e.p.*, remains of epidermal cells; *den. c.*, outer dentine cells of the papilla. No dentine is yet deposited. × 400.

the cells. The hardest part and outer layer of the crown, the enamel, is elaborated by these cells and secreted at their proximal ends. These

cells are destroyed as the crown of the tooth, supplied with its enamel, emerges from the dental groove.

With the evolving of this enamel tissue the papilla grows and assumes the shape of a mature tooth (Figs. 256 and 257). The mesenchyme becomes differentiated into an inner and an outer zone of cells. The inner zone gives rise to the mass of connective-tissue elements known as the pulp. This pulp contains stellate connective-tissue cells, connective-tissue fibrils, and a semifluid inter-fibrillar substance. The pulp supports the nerve and blood supply of the tooth. The outer zone of mesenchymal cells becomes pyriform, with their small ends radiating from the axis of the tooth (Fig. 258). These elaborate at their distal ends the hard part of the tooth known as the *dentine*. They are called *odontoblasts*. Numerous fine canals traverse the layer of dentine.

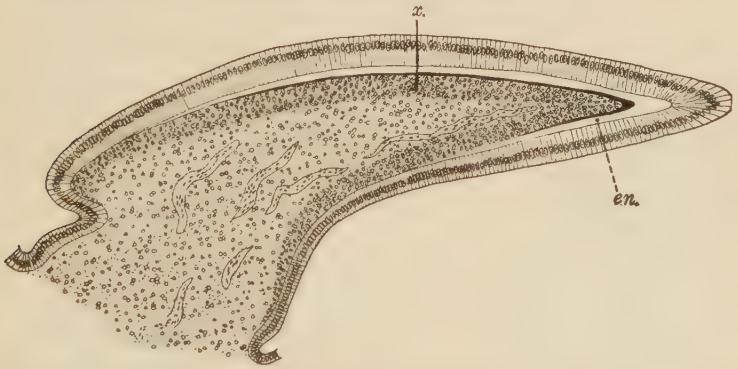

FIG. 257. — Half-matured tooth of same animal; blood well into the papilla, whose outer edge is hardening into the dentine. *en.*, enamel laid down by the tall columnar epithelium. *x*, plane of section shown in Fig. 258. × 55.

Into these canals or canaliculi the odontoblasts send protoplasmic processes. These processes take part in the calcifying of the dentine. This calcification takes place first at the periphery where the processes end. As calcification advances the protoplasmic processes of the odontoblasts retreat, and the canaliculi are almost obliterated. The root of the dogfish's tooth is not developed to form a fang. It is a perforated plate rather than a tube. It is composed of a dentine wall without enamel and a pulp cavity. The nerve and blood supply enters the tooth through this root.

Accessory digestive glands. **Calcium-carbonate glands.** — These glands occur in the earthworm. Externally they appear as three pairs of pouches or diverticula from the side of the œsophagus. The first pair of diverticula lie in the tenth segment. These are the most prominent. The second and third pair lie in the eleventh and twelfth segments respectively. In section the first pair is clearly seen to be an

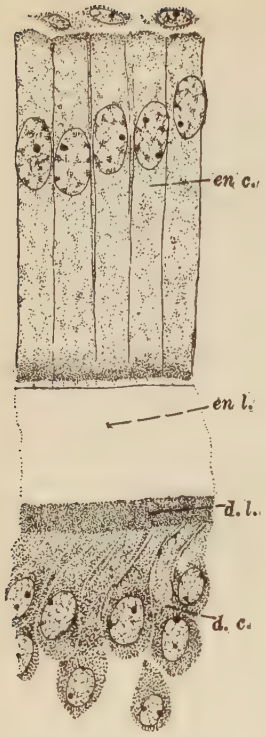

FIG. 258. — Details of last figure at *x* enlarged. *en.c.*, enamel cells; *en.l.*, enamel layer; *d.c.*, dentine cells; *d.l.*, dentine layer. × 700.

invagination of the wall of the œsophagus. These first diverticula are lined with columnar epithelium differing but little from the epithelium of the œsophagus. These sacs are mere storehouses for the products of the true glands. The second and third pair of diverticula are but pairs of swollen regions in a single pair of glands. Each gland extends from the fourteenth segment anteriorly to the tenth, where it opens into one of the first pair of diverticula. Such a gland consists of a number of flattened tubes with relatively wide lumina. These tubes lie longitudinally between the epithelium of the œsophagus and the layer of circular muscles. The glandular tubes have in transverse section the outline of truncated wedges; these flattened glands lie with their flattened sides radiating from the œsophagus. They are of mesenchymal origin. Each tube is coated with a *membrana propria* or basement membrane. The wall of the tube is formed by a deep syncytial layer. The cytoplasm of this syncytium is alveolar in its appearance. The inner margin of the cytoplasm varies with the functional periods. Likewise the nuclei, which arise by division near the membrana propria, vary in size, shape, and position. The nuclei when young are oval and lie near the membrana propria, with their long axes parallel to the base of the syncytium. In the height of their development the nuclei are spherical and lie near the middle or towards the margin of the cytoplasm. The secretion of lime takes place near the margin of the cytoplasm. In the elaboration of lime or calcium carbonate both the marginal nuclei and the cytoplasm disintegrate (Fig. 259).

Mucous glands from the base of the tongue of a bat will serve to show an accessory alimentary tissue used for lubrication. Each of these glands is a branching tubular gland. Its cells are tall columnar elements measuring

FIG. 259. — Section of a portion of epithelium from the calcium carbonate glands of the earthworm, *Lumbricus*. (After HARRINGTON.)

about 9 by 25 microns. Their cytoplasm has been inflated by a secretion mass so as to form a capsule with but a film of cytoplasm for a wall. The small, rounded nucleus, more or less distorted by the pressure of the secretion substance, lies at the base of the cell in the thickest part of the cytoplasm. The secretion mass is mucus. Fresh mucus is a viscid, cloudy substance. In all specimens fixed in formalin, alcohol, or acids it appears as a dense, floccular mass which does not stain readily in acid stains (Fig. 260). This cell type is characteristic of all mucous tissues, except that where the mucous cells are scattered they are enlarged distally and are called goblet cells.

FIG. 260. — Part of the secreting epithelium from a mucous gland in the base of the bat's tongue. *r.c.*, resting cell (young cell?). × 700.

Conductive tissues. — **The œsophagus of the squid** is a tube with a thick wall. The outer coat is composed of connective tissue covering a heavy layer of circular smooth muscle fibers inside of it. Beneath this is a longitudinal layer of smooth muscle. A connective-tissue submucosa supports the inner layer, which is composed of very low, stout, columnar, epithelial cells. Each cell has a finely granular, homogeneous cytoplasm and an oval nucleus. These cells elaborate a tough cuticle (Fig. 261) which is probably elastic. When not in use, the whole membrane lies in longitudinal folds.

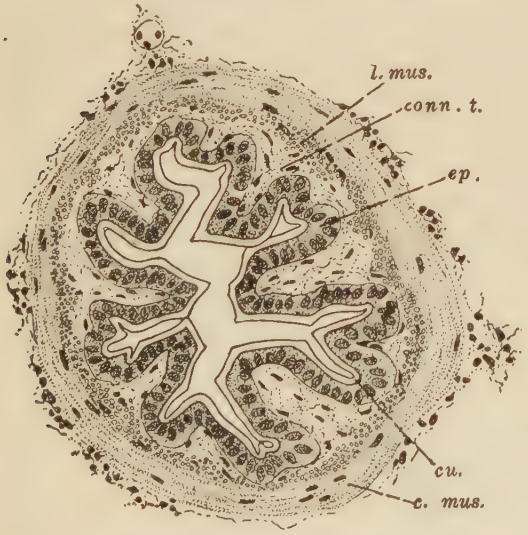

FIG. 261. — Transverse section of œsophagus of squid, *Loligo Pealii*. *cu.*, cuticle; *ep.*, lining epithelium; *conn.t.*, connective tissue; *l.mus.*, longitudinal muscle fibers; *c.mus.*, circular muscle layer. × 200.

The œsophagus of the cat has also a thick wall composed of two muscular layers, a connective-tissue submucosa, and an epithelium. It differs from that of the squid in having the longitudinal muscles outside the circular muscles. The more significant difference, however, is in the epithelium. A cuticle is not developed; instead the

epithelium is greatly stratified (Fig. 262). The dead, outer cells resist abrasion as the cuticle does in the œsophagus of the squid.

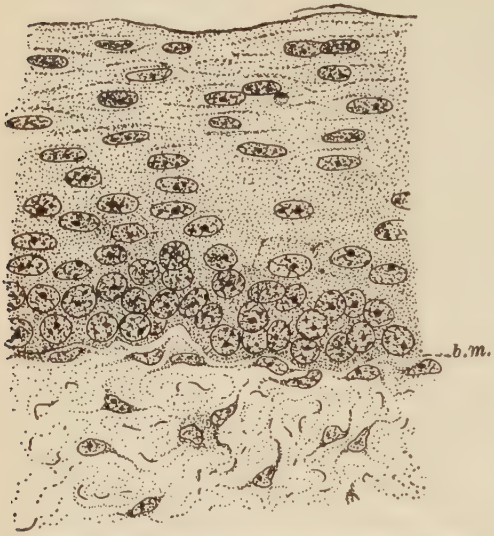

FIG. 262. — Part of a transverse section of the stratified, epithelial lining of a cat's œsophagus. *b.m.*, basement membrane. × 700.

Absorptive tissue. — **The lumen of the small intestine of a pigeon** bears numerous villi. Each of these has a median, connective-tissue frame-work — the *mucosa.* Within the mucosa are nerve fibers, lymphatic vessels, and blood vessels. These vessels are conspicuous, and it is into them that the assimilated products are emptied (Fig. 263). The villus is covered with a columnar epithelium. There are scattered goblet or mucous cells in this epithelium. These are not concerned with the absorption of food. The cells most numerous in this epithelium are tall cells with oval nuclei lying in the lower third of each cell. The free or distal end of each cell is marked by a dense cuticular border which cannot, however, be compared with a real cuticle.

Lymphocytes are said to aid in the absorbing of fats as emulsions. Two of these cells are shown lying between the absorbing cells in Figure 263. Others travel through the epithelium to lie in the lumen of the canal. These cells are supposed to receive the fatty emulsions from the absorbing cells, and then find their way back to the lymphatic vessels where they disintegrate and discharge their contents. These cells are not confined to the absorbing tissues, but are found in the epithelium of the stomach as well. They have a great affinity for

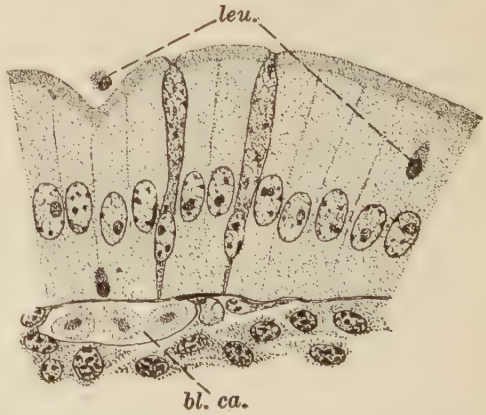

FIG. 263. — Portion of a longitudinal section of a villus from the pigeon's duodenum, showing several absorbing cells and two mucous cells. *bl.ca.*, blood capillary with blood cells inside; *leu.*, lymphocytes. × 870.

eosin, and are best demonstrated by the use of this stain in combination with others.

Digestive tissues. — **The digestive tissues in** *Cerebratulus* are not highly

FIG. 264. — Portion of the digestive epithelium of *Cerebratulus lactatus*, showing a deep mucous cell. *n.*, nucleus of mucous cell. × 1200.

differentiated. In the œsophageal epithelium the same elements are represented as are found in the intestine. In this region there are two types. The columnar, ciliated cells have oval to rounded nuclei lying in the basal third of the cell. There is an occasional vacuole bearing a secretion substance found within these cells (Fig. 264). The serous cells in this region are more than twice as large as the ciliated cells. They extend far below the basement membrane. The nuclei are small and lie quite near the base of the cells. The secretion bodies stain black in iron hæmatoxylin. In the intestine these elements become much taller, so that the intestinal epithelium is very thick. The ciliated cells here are as tall as the albumen cells. Figure 265 shows but the lower third of these cells. The ciliated, secreting cells are most numerous in the dorsal region of the intestine. Their cytoplasm contains many spherical secretion particles. The elliptical nuclei lie near the base of the cells (Fig. 265, *A*). The albumen cells in this region are scattered and very slender. Their secretion particles are small, round bodies (Fig. 265, *C*). On the ventral side of the intestine the albumen cells are the more numerous. In

FIG. 265. — The bases of three long digestive cells from the enteron of *Cerebratulus lactatus*. *nu.*, nuclei; *b.m.*, basement membrane. Cells filled with secretion granules. × 1200.

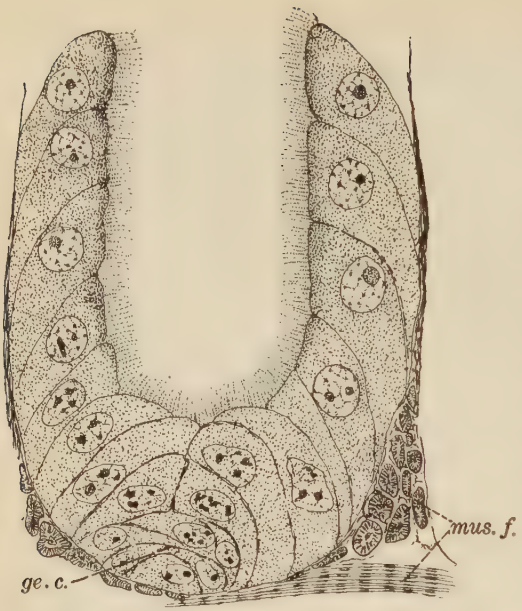

FIG. 266. — Lower part of one of the fold-glands in the intestine (ventriculus) of a hornet, *Scolia dubia*. *ge.c.*, germinal cells; *mus. f.*, muscle fibers in longitudinal and transverse section. Upper cells are absorptive. × 750.

this region they have greatly increased in diameter. The secretion particles of these cells are large and oval (Fig. 265, *B*).

The epithelium of the intestine of the hornet may be taken as an example of digestive tissue among the insects. The epithelium is thrown into wavelike folds or corrugations. The columnar cells vary in height. They are tallest on the ridges and smallest in the grooves. The fundus of each groove forms a center from which new cells appear to be proliferated. The cytoplasm is reticular to alveolar. The nuclei lie near the bases of the cells (Fig. 266).

We shall take the **gastric gland of the crayfish** as an example of the more highly specialized digestive tissues of the Arthropoda. This

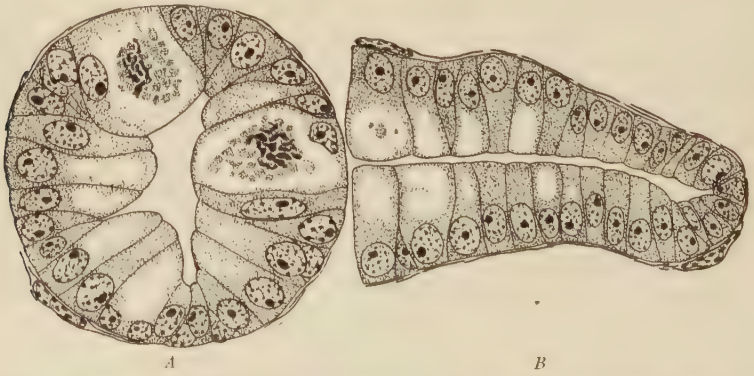

FIG. 267. — *A*, transverse section of the middle part of a tubule of the crayfish's digestive gland; *B*, longitudinal section of the tip of same showing the growth point. Many vacuoles shown with or without contained secretion products. × 1000.

gland is composed of a very extensive system of tubules. Each tubule is lined with a columnar epithelium which is furnished with a membrana

propria, or basement membrane. The wall of the tubule is relatively thick and incloses a narrow lumen. In this case the cells are proliferated at the fundus of the tubules and at certain regions along the sides. When immature they are small with compact, dense protoplasm. The nuclei are oval to slightly irregular in shape. The cells and their nuclei increase in size. The cytoplasm becomes vacuolated at the distal ends. Within these vacuoles secretion, and perhaps certain excretion, products appear (Fig. 267, *A* and *B*). When the cell is fully grown it has attained a great size, and bears an immense vacuole which crowds most of the cytoplasm and the nucleus to the base or one side of the cell (Fig. 267, *A*).

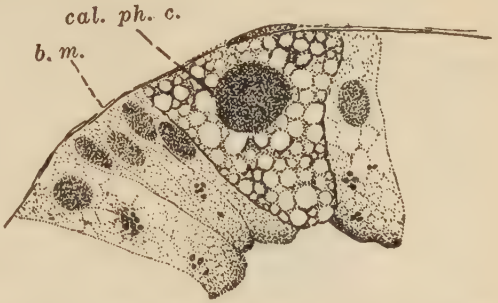

FIG. 268. — Cells from the digestive gland of *Mesodon* (*Helix*). *cal.ph.c.*, calcium phosphate cell. Others are hepato-pancreatic cells. *b.m.*, basement membrane, on which lies a narrow connective nucleus. × 970.

The **general digestive epithelium of mollusks** is strongly ciliated (see Fig. 52). In the connection with this ciliated tissue **gastric glands** have been developed. As an example of this more highly specialized tissue we shall take the so-called hepato-pancreatic gland of *Mesodon*. The chief cells of this greatly branched gland are columnar cells, as shown in Figure 268. These cells secrete a ferment that aids in digestion. They have also the power to elaborate glycogen. In addition to the chief cells an occasional albumen cell and as frequently a calcium phosphate cell is met with.

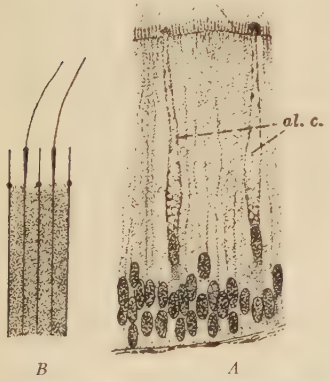

FIG. 269, *A* and *B*. — *A*, digestive epithelium from intestine of *Amphioxus*. *al.c.*, albumen cells which bear no cilia. × 1250. *B*, enlarged ends of the same sort of epithelium to show relations of cilia. × 3000. (*B* is after SCHNEIDER.)

The **digestive tissues of *Amphioxus*** are but little specialized. The intestinal epithelium is carried into the hepatic cœcum or gastric gland. This epithelium is composed of very slender ciliated cells. Each cell according to Schneider's figure bears a single cilium. Occasional albumen cells are found lying among the ciliated digestive cells. The nuclei of these cells lie farther from the basement membrane than do the nuclei of the ciliated digestive cells (Fig. 269).

Pancreatic tissue. — **The pancreas of a frog** is a compound tubular gland. The tubules are incased by a delicate membrana propria, and are held together by a connective tissue, which carries the nerve and vascular supply. The wall of the tube is formed by simple columnar epithelium. The cytoplasm of a pancreatic cell bears a variable number of fibrils, some of which are always found in the basal third of the cell. The distal end of the cell is usually granular and filled with spherical secretion particles. The nucleus always lies in the cytoplasm bearing these fibrils. There is frequently found in a pancreatic cell a round, dense body lying near the nucleus, called the paranucleus or nebenkern. This has been interpreted by Mathews as a tangle or knot of the fibrils that are commonly found in the pancreatic cells (Fig. 270).

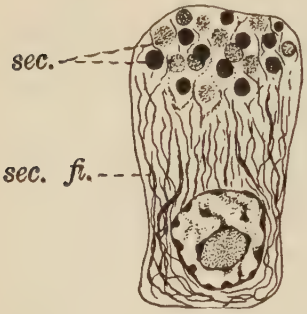

FIG. 270. — Pancreas cell from salamander. *sec.*, secretion substance; *fi.*, secretion fibrils. (After MATHEWS.)

Gastric tissue. — **The gastric glands of a muskrat** are tubular. The glands are supported in the *mucosa* of the stomach. These glands open through depressions or crypts into the stomach. Each gland is incased in a membrana propria. The glandular epithelium is composed of two types of cells. The smaller and more numerous ones are small columnar cells. The cytoplasm is rather dense; the nucleus is round and located near the center of the cell. They much resemble serous cells. They receive the name of *chief cells* (Fig. 271, *A*, *c.c.*). These cells elaborate a digestive fluid which is active in an acid medium. Crowded back by the chief cells and lying more remote from the lumen of the tube are larger cells which secrete hydrochloric acid. These are known as the *acid cells*. An acid cell is usually rather large. Its original form was columnar, but in most cases the acid cell has a shape conforming to its position. The cytoplasm is reticular in appearance or highly vacuolated, and is not so dense as the cytoplasm of a chief cell or a serous cell. The nucleus is spherical and centrally placed. Although lying remote from the lumen, there is always a passage between the chief cells for the secretions of the acid cells to pass out (Fig. 271, *A*, *a.c.*). In the birds these two types of cells form different glands, so that there are glands lined with chief cells and others with acid cells. These glands lie in different parts of the enteron. The acid glands are always anterior to the glands with the ferment-secreting cells. The latter are tubular glands that dip into the *submucosa* of the gizzard (see Fig. 251). The acid glands are found in the proventriculus. Each gland is a compound gland. There is a central tube which is lined with an epithelium

but little differentiated from the epithelium lining the lumen of the proventriculus. From this axial primary tube many secondary tubes radiate

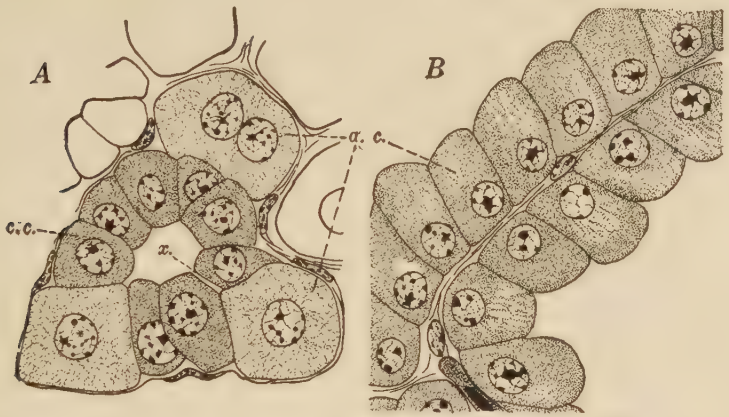

FIG. 271. — *A*, transverse section of gastric gland in stomach of muskrat, *Fiber zibethicus*. *c.c.*, chief cells; *a.c.*, acid cells; *x*, cleft between chief cells through which the secretion of the acid cells passes into the lumen. *B*, parts of the walls of two adjoining acini in the proventiculus of the pigeon, *Columba*. The epithelial cells are acid cells exclusively.

along its entire length and its fundus. The secondary tubes are structures with spacious lumina. They empty into the axial tube which serves as an excretory crypt or duct for the compound gland. The epithelium of a glandular tube is supported by a *membrana propria*. It is composed of large acid cells which are not generally crowded, hence are columnar. In regions where a cell is crowded by its fellows it takes the triangular form of mammalian acid cells (Fig. 271, *A*, *a.c.*).

Serous tissue. **Serous tissue of a gland from the base of the bat's tongue.** — This is a branching tubular gland. The cells when quite active are greatly distended and columnar or even pyramidal in form. They are rather small, measuring about twenty microns in height and less in width. The cytoplasm contains numerous small excretion granules which are most numerous and largest at the distal ends of the cell. The nuclei are oval to rounded. They lie near the center of the cytoplasm for the most part in the lower third of the cell. Tubules bearing cells distended with secretion particles have small lumina (Fig. 272). When the cells have given off their secretion contents, they shrink and thus form a large lumen in the tubule.

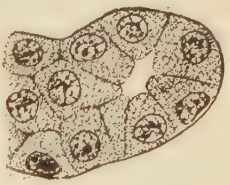

FIG. 272. — Acinus of a serous gland from the base of the tongue in a bat. To show the serous cells. × 700.

Hepatic tissues. — **The liver of** *Cryptobranchus* **must be considered a**

tubular gland. The branching has been so extensive that the lumina which constitute the so-called *bile capillaries* have been very greatly reduced and branched. These bile capillaries may have a wall of five or six surrounding cells or of but two cells. The hepatic cells are cubical to polyhedral. Their faces approximate to form the lumina or *bile capillaries.* The bile capillaries send intra-cellular branches into the cytoplasm of the cells (Fig. 273). The cytoplasm is seen to be vacuolated when free of glycogen and pigment granules. The nuclei are spherical and lie near the center of each cell. Chromatin is very conspicuous in these nuclei. It forms large deeply staining bodies suspended upon a lining reticulum; a spherical nucleolus is usually to be seen within the nucleus (Fig. 274). Canals in the cytoplasm empty into the bile capillaries. The bile capillaries converge to form *hepatic ducts*, which lead off from the lobes of the liver to the bile duct.

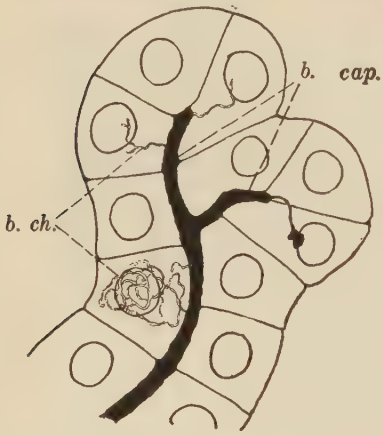

FIG. 273. — Golgi preparation of a portion of frog's liver. Cells and nuclei in outline. *b.cap.*, intercellular bile capillary; *b.ch.* intra-cellular bile channels. × 750.

Technic. — These tissues are easy to handle for general work and very few special processes are necessary. Owing to the presence of food material in the cavities, and especially to the fact that the tissues themselves secrete digestive juices from which they are immune only as long as life exists, it can be seen that they must only be taken from the freshest of newly killed animals and must be immediately placed in the fixative. This, however, often causes another trouble, the contraction and shrinking away of the still living inner parts from the already fixed and stiffened

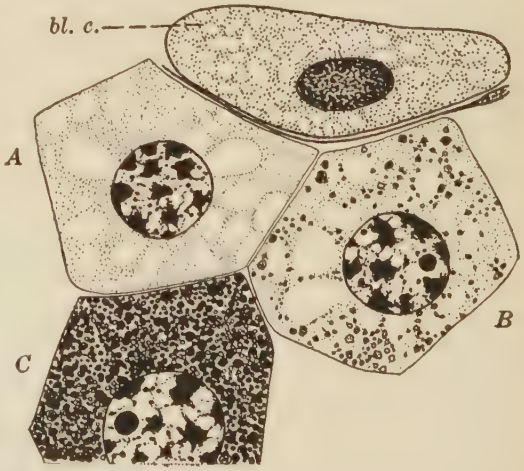

FIG. 274. — Three liver cells from the salamander *Cryptobranchus*. They show, in *A*, *B*, and *C*, three successive stages of pigmentation. *bl.c.*, blood cell.

outer epithelia. This almost always occurs in the villi of the mammal intestine. It may be avoided by several devices, as the injection of the fixative through the blood channels after the use of amyl nitrite. Also by the complete killing of the tissue by the use of chloroform.

The tissues are sometimes very delicate and susceptible to handling. These fragile parts may often be fixed *in situ*, especially by the use of fixatives containing formalin. Chrom-aceto-formol is one of the best for this purpose. Gentle injections into the lumen and into the surrounding cavities should be made.

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CHAPTER XVI

THE DUCTLESS GLANDS

In the developing embryos of vertebrate animals the endodermal epithelium of the pharynx is invaginated into a number of paired or median clefts or pits which, in most cases, are later cut off from communication with the digestive lumen by the constriction and atrophy of the connecting ducts. These structures may be classified as *ductless glands*, although in some of them a duct persists. They play an important part in the animal's economy, as can be readily, although negatively, demonstrated by experiment. Their structure also shows that there must be considerable specialization in this function.

The most anterior of these structures is partly derived from a pit in the dorsal wall of the mouth. This invagination unites with a corresponding invagination of the wall of the diencephalon or mid brain to form the *hypophysis* or *pituitary gland*. Further back are to be seen the five gill clefts which occur in all vertebrate embryos as five invaginations of the pharyngeal epithelium. They sometimes do, and in other cases do not, open through the sides of the neck to the exterior. In the mammals they do not so open and, while the first pair form no glands, the second pair form the paired *palatine tonsils*, whose ducts persist; the third pair form another ductless gland called the *thymus gland*. From a median invagination of the epithelium on the base of the tongue is derived the *thyroid gland*, and the fourth and fifth gill clefts probably give rise in the same way to the *parathyroid bodies or glands*.

The *adrenal gland* originates in an entirely different manner from two kinds of tissues, and is a ductless gland in the body cavity. It, too, is of vital importance, as is attested by the death of the subject from which the adrenals have been removed. The *carotid gland* and *coccygeal gland* are two small ductless glands found on the carotid artery, and ventrad of the middle sacral artery respectively, in man and some of the mammals.

All these glands discharge their secretions into the blood as well as take their food materials from it. They therefore have a very incomplete lumen which rarely remains in communication with the exterior (as in the tonsils) and is usually cut off from it (as in the thyroid, thymus,

etc.), or they have no lumen at all (as in the adrenal, etc.). We shall take up the descriptions of these tissues in the order in which they are mentioned above.

Several important secreting tissues are associated, in the vertebrates, with the walls of the brain. In earliest embryonic life the lower brain wall is invaginated to form a longer or shorter ventral depression, the infundibulum, whose lower end becomes cut off to form the hypophysis. The posterior wall of this infundibular invagination remains thin, and becomes of a complicated sac-shaped form, in some animals with the secretory epithelial layer lining a lumen that remains in connection with the brain cavity, while the proximal surface of this epithelium is brought into contact with an abundant blood supply in sinusoids. This structure is called the *infundibular gland* or, less properly, the "saccus vasculosus."

An invagination of the roof of the oral cavity of the embryo arises, and reaches up until its fundus comes into a close relation with the hypophysis. The fundus of this invagination becomes cut off and develops into a mass of cellular cords, being known as the *glandular lobe of the hypophysis.*

The *neural lobe* of the hypophysis generally consists of a fibrous medulla and a cortical region of cells which may have some glandular function, although none has been actually determined.

We shall first pay particular attention to the infundibular gland as found in the lower vertebrates and then to the glandular lobe of the hypophysis as seen in one of the higher forms.

The infundibular gland of a flounder, *Pseudopleuronectes Americanus*, begins in the young embryo as a sac-like invagination of the posterior wall of the infundibulum. The walls of this sac, therefore, consist of an epithelium which was previously invaginated from the ectoderm to form the brain and would otherwise have become nervous in function. This sac-like invagination becomes folded and bent by many septa that arise from its walls. With each fold there comes a blood-vessel loop which enlarges to become a thin-walled sinusoid. The whole number of vessels soon form a plexus that carries a large quantity of blood and gives the structure its characteristic red color during life.

Figure 275 shows parts of two rows of the secretory cells and parts of the blood spaces upon which they lie. These latter are filled with the typical nucleated corpuscles of the fishes. The cells are large and heavy with a dense cytoplasm that shows no vacuoles or granules, or other evidences of activity. The nuclei are round, and placed slightly toward the proximal end of the cell. The nucleolus lies almost exactly in the center of the nucleus, and the chromatin is arranged in strands which radiate with some regularity from the nucleolus to the nuclear mem-

brane. The distal end of such a secreting cell ends in a knob-like process directed into the gland lumen (which is part of cavity openly connected with the brain lumen) and a number of peculiar, heavy granules, some of which seem hollow, are collected around this knob like a sort of cap.

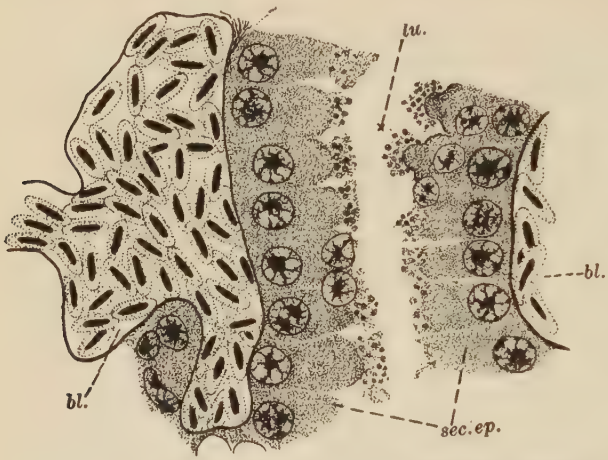

FIG. 275.—Part of a section through the infundibular gland of a flounder, *Pseudopleuronectes Americanus*. *lu.*, lumen; *bl.*, blood vessels; *sec.ep.*, secreting epithelium. × 750.

A rather remarkable feature is the number of slightly smaller nuclei that lie in this epithelium near the distal ends of the cells. They appear to be in the secretory cells or between them. Well-preserved preparations show that they belong to smaller cells which lie between the ends of the secretory cells and seem to be degenerating in the mature gland. No evidences of any renewal process can be seen that would indicate such a casting off of cells, however.

This gland evidently secretes some substance into the brain-cavity fluids. It is not the only point, however, at which the brain-wall cells have given up their nervous functions to become gland cells. At other points in the brains of all vertebrates are found places where the wall has been evaginated into long, branching, and anastomosing tubes of simple epithelium, and blood vessels have followed these tubes in and occupy their centers. Thus again the blood is brought into relation with the brain-cavity fluids with only a simple epithelium and the thin blood-vessel endothelium lying between them. It is considered in this case that the epithelial cells are active in removing substances from the brain-cavity fluids. Such a structure is known as a *choroid plexus*.

The glandular lobe of the cat's hypophysis will next claim our attention. As was said above, this is an epithelial invagination of the mouth cavity which has become subsequently cut off, and superficially attached to the neural lobe of the hypophysis. It undergoes a complex development in many animals, and comes to be made up of lobules like the thyroid gland (see below). In the cat, however, the lumen almost dis-

appears, and the tissue (Fig. 276) appears as a series of compact cords of cells, among which pass many small blood vessels. This blood supply is not as abundant as it was in the infundibular gland.

A principal point to be observed is that the cells are of two kinds, a larger cell filled with a purely granular secretion, and a smaller cell that does not stain in the same way and is not filled with a secretion. These may be two varieties of cells, or they may be different physiological stages of the same kind of gland cell. They have been designated by the manner in which they take up different anilin dyes as the *chief cells* and the *chromophilic cells.*

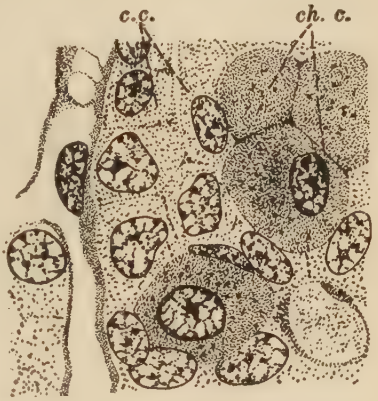

FIG. 276. — Small portion of tissue from the glandular lobe of a cat's hypophysis. *c.c.*, chief cells; *ch.c.*, chromophilic cells. × 875.

The palatine tonsils of an oppossum, *Didelphys*, may be considered here because of their origin, and also because they act, in a manner at least, as ductless glands, although the invaginations from which they are derived remain in open communication with the digestive lumen. Each tonsil consists of a slightly raised area from which several *crypts* have been formed by invagination. The stratified epithelium of the oral cavity is continuous through these amplifications, but becomes thinner at the bottom of each infolding.

Beneath the epithelium is a thick, mesodermal layer composed of connective tissue infiltrated by lymphatic cells. Lymph nodules or germinal centers are found through this mass at regular intervals, and they are typical of lymphatic tissue in every way. As a rule, the lymphatic tissue is but one nodule thick. In many forms, however, it is apparently thicker, owing to a greater involution of the epithelium.

It is in the relation of this lymphatic tissue to the invaginated epithelium that the tonsil possesses a specific character which allows one to speak of it as a "gland." Some of the amœboid lymph cells are constantly forcing their way distally between the epithelial cells, and finding their way out of the body into the digestive tube. They are numerous in the saliva and mucus, and possibly act as scavengers and destroyers of bacteria. They pass through the epithelium in greater numbers during certain conditions of the body, and they also come through certain parts more than they do through others.

The illustration (Fig. 277, *A* and *B*) shows two small portions of the epithelium which lines one of the several tonsilar crypts of the opos-

sum. A small amount of the underlying lymphatic tissue appears, and lymph cells are seen passing through in greater numbers in the right-hand figure. They almost take away the characteristic appearance of the epithelium at this point, making it look like lymphatic tissue instead. A thin, distal layer of stratified cells persists in nearly all stages, being renewed, when the leucocytes have destroyed it by passing through in large numbers.

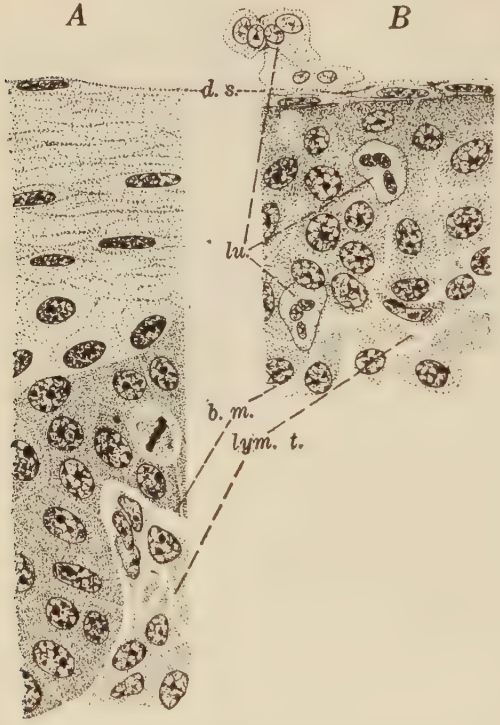

FIG. 277. — *A* and *B*, epithelium from two regions in the tonsilar cleft of a young opossum. *d.s.*, distal surface of the stratified epithelium; *b.m.*, basal membrane of epithelium; *lu.*, leucocytes crawling through epithelium and in lumen; *lym.t.*, lymphatic tissue beneath the epithelium. × 650.

There are many other places in the digestive tract in which lymphatic tissue is put in this relation with the enteric lumen. Sometimes a simple epithelium forms the surface through which the leucocytes break. Where there is but little of the adenoid tissue, there are no lymph nodules to propagate a new supply of the lymph cells, and these are brought or wander in from other germinal centers instead. Amœboid cells resembling leucocytes are always to be seen between the columnar cells which line the larger part of the enteron. At other places the lymph tissue is collected locally into isolated masses which, when large enough, are found to contain a germinal center, and here the amœboid cells break through the epithelium *en masse*. Such are the Peyer's patches of the small intestine.

Another pair of embryonic gill clefts, the third, invaginate as did the tonsil glands to form another, and temporarily larger, gland which is called the *thymus gland*. It is a much more specialized tissue than the tonsil, and grows rapidly until a rather early period in life, after which it undergoes a retrograde development, and finally degenerates in age by a fatty transformation. Its structure and development are difficult

to follow, and have been the subject of much study and controversy. The two most distinctly formulated views of its origin are, first, that it is an epithelial invagination to which has been added a mesodermal reticulum in which many leucocytes and other more specialized cells have emigrated; and secondly, that it is a purely epithelial structure, some of whose rapidly multiplying epithelial cells have formed a reticulum, while others have become specialized into several kinds of cells, the larger number of which closely resemble leucocytes. Many adherents of this view approach a mean by saying that true leucocytes of mesodermal origin do move in among the thymus cells, especially in the outer or cortical part. They sometimes carry this idea to the extent of a third theory, which claims that the cortex only of the lobules is mesodermal in origin.

Upon examining **the thymus gland of a cat** at about the time of birth, we find the gland is composed of a fairly large number of solid, cellular lobules which each exhibit a rather weakly defined cortical and a medullary region. These lobules are packed closely together to form an elongated mass of tissue, and the medullary portion of each, on the inner side of the lobule, is continued through the cortex as a cord of tissue which emerges to unite with the similar cords from other lobules and thus form a central connecting mass similar to the medulla of each lobe. Light coverings of connective tissue surround each lobule and carry a heavy blood supply into its tissue.

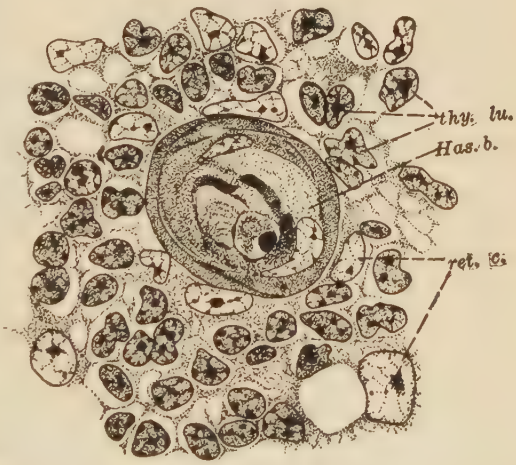

FIG. 278. — Small central portion of a lobe of the thymus gland of a kitten. *Has.b.*, Hassall's body; *ret.c.*, reticular cells; *th.lu.*, thymic leucocytes. × 800.

Under a high power (Fig. 278) the gland appears to be a reticulum of connective-tissue cells in whose meshes there lie very many lymph cells and other cells. As shown by Hammar, this reticulum originated from a lumenless and ductless, invaginated mass of epithelial cells from the region of the third embryonic gill cleft. This mass became reticular by the formation of processes, from the cytoplasm of its cells, which remained united with the processes from other epithelial cells. By the lengthening of the connecting strands thus formed a fairly wide-meshed reticulum is produced.

This reticulum is the basis of structure in the thymus tissue of young animals, and in its meshes lie the other cells. These are the *thymic lymphocytes*, the *myoid cells*, the *Hassall's cells*, and the several types of *ciliated* or *bordered cells* found more commonly in the thymus of lower vertebrates but also seen in the mammals.

The thymic lymphocytes are of several varieties and least is known of them, in regard to their origin and function, on account of the difficulty of study which they possess in common with other lymphoid tissues, the movements of their cells which cannot be followed in life but must be studied by successive stages in a series of different fixed sections.

The myoid cells are evidently derived by a direct specialization of some of the elements of the epithelial reticulum. These increase in size and the cytoplasm shows a distinct fibrillation. The fibrils are parallel

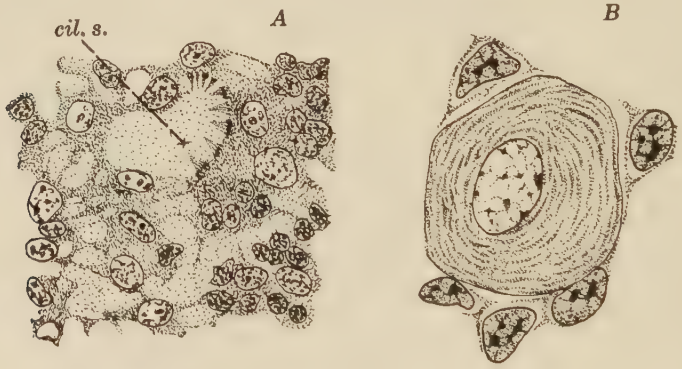

FIG. 279. — *A*, portion of thymus tissue from an infant. Shows a ciliated space (*cil.s.*) (After HAMMAR.) *B*, myoid cell from thymus of a small pickerel frog. × 850.

and the groups form circular or whirlpool masses which center after a fashion around the nucleus. These fibrils show a muscle-like segmentation in some animals, as the frog (Fig. 279, *B*). The change may stop here or may go on to the development of long strands of cytoplasm in which the fibrils run parallel for some distance and show a strong segmentation into anisotropic and isotropic segments that correspond to those of muscle.

Certain of the cells of the epithelial reticulum, situated always in the medullary substance, become enlarged and grouped into concentrically arranged masses which are known as "Hassall's bodies" from their discoverer (Fig. 278). At first small and solid, these bodies increase in size and the central portion degenerates and breaks down. The several concentrically arranged and stratified peripheral layers show many characteristics of a stratified epithelium, and it was considered by many histologists that these hollow bodies were the remnants of the epithelium

which lined the original invagination that produced the thymus. This view is incorrect, in so far as the original invagination was not hollow, but solid, and therefore had no lining stratified epithelium. On the other hand, this original invagination was composed of what would otherwise have become stratified cells, and as some of these cells in the central position give rise to the Hassall's cells, it might be argued that these bodies represented a late and imperfect attempt on the art of the gland to develop a lumen lined by a stratified epithelium. The process might be compared with other invaginations which only develop their lumen after their structure is well under way, as in the invaginated nervous tube of some teleost fishes.

Another remarkable development derived from the epithelial reticulum of the thymus gland is seen in the form of small openings in single cells, or formed by groups of cells, some of which develop cilia or cuticular edges on such of their surfaces as bound these openings (Fig. 279, *A*). The cilia are well formed and must undoubtedly be active during life. The cavities in which they work are closed and often contain irregular masses of some unknown secretion product. These ciliated openings apparently have no function in which ciliary motion can bear a necessary part, and they may be looked upon in the same light as Hassall's bodies, as vestigial lumina which are lined with ciliated epithelium instead of stratified. The few well-defined mucous cells found in the tissue must also be regarded in the same light until some more definite function can be proved to require them.

The remaining and largest number of cells of this tissue are the apparently amœboid cells that resemble leucocytes of several varieties. That some of these are derived from the mesoderm is undoubted. That any of them are transformed epithelial elements is very improbable. They have probably been acquired by the moving in of real lymph cells. Their slight differences in structure and staining reactions are partly responsible for the difference in texture between the cortex and medulla of the lobules.

We next shall examine **the thyroid gland of a fish**, *Raja lævis*, in which the organ is typically developed.

This organ consists of a series of lobules lying side by side in a mass of vascular connective tissue. Each of these lobules is hollow and is lined by a simple epithelium which is cuboidal in the smaller ones, and high enough to be called columnar in others. The nuclei are round and full and lie slightly proximad of the cell in the smaller lobules and well toward the basement membrane in the larger ones (Fig. 280). The cytoplasm is clear and shows several sorts of granules that probably represent different stages in the elaboration of the secretion. The cells have been divided into two classes on account of constant differences

in the presence of one or the other of these different kinds of granules. The cells pass the secretion into the lumen of the lobule, which has no duct or means of discharging the mass externally. It is retained as a colloid substance in the lumen of the lobule. There is a constant growth of the younger lobules, which appear at certain germinal centers and slowly increase in size. Figure 280 shows several of the cells which line one of the larger lobules in this fish.

FIG. 280. — Vertical section of a small part of the epithelium which lines one of the lobules of the thyroid gland of a skate. *sec.*, secretion in lumen; *bl.v.*, blood vessel in connective tissue that surrounds the lobule. × 1100.

The thyroid gland is usually accompanied by another structure, **the parathyroid gland or body.** In most of the higher vertebrates the parathyroid appears as several small irregular bodies, the lobes, which lie on the posterior edge of the thyroid. They vary in form and position, and may be two or four in number according as one or both of the last two pairs of gill clefts took part in their formation. Each lobe of this tissue is invested with a dense connective-tissue sheath, and its interior reticulum of loose connective tissue is filled with two kinds of cells which appear to be specific to the gland. The most abundant of these are called the *principal cells.* They are slightly irregular ovals or spheres, with a large

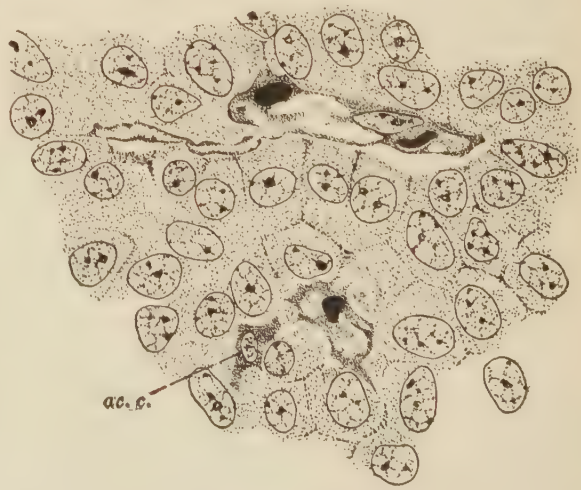

FIG. 281. — Bit of tissue from the parathyroid gland of a young cat. Two blood vessels are seen, near one of which is a single acidophile cell (*ac.c.*). × 750.

round nucleus whose chromatin forms a peculiar pattern. These cells form various cords, groups, or even acini (Fig. 281). In some of them the cells appear to be surrounding a small lumen that contains a colloid substance like the thyroid substances. Besides these principal cells there is another kind of much the same character, but containing a smaller and deeper staining nucleus. This stains in acid dyes best, and the cells are known as the *acidophile cells*.

The blood supply of this tissue is very rich. It consists of an arterial system which percolates through the tissue in large, thin-walled vessels which empty into veins to return to the central, circulatory organ. Between these blood vessels and the gland cells are found but few strands of connective tissue. The vessels are lined by a single, thin endothelial layer.

Two important secretory tissues are found, in the vertebrate body, which pass their secretion into the blood, where it is of importance to the organism's economy. Their constant presence in the neighborhood of the kidneys has given them the general designation of *renal bodies*.

One of these bodies is composed of cells that originated by a development from cells that otherwise would seem destined to become sympathetic nerve cells. Instead of acquiring nerve processes and neurofibrils as well as perceptory and motor end-organs, they acquire a secretory power and (to the eye) a peculiar texture which can best be noticed by placing them in chromium salts, which they take up more readily than other cells, and which stains them a dark brown.

The secretory power results in the production of an organic substance called "adrenaline," which, when injected into the circulation of the same or other vertebrate animals, causes a contraction of the blood vessels and a consequent rise in blood pressure. These cells are most commonly called the *chromaffine cells*, and they form the *paraganglionic bodies*, as this sort of tissue has been called. Chromaffine cells may be developed in other situations than in the renal bodies, and perhaps from other cells than young nerve cells. The function of such cells would appear to be the same. Many isolated chromaffine cells also appear in otherwise purely nervous, sympathetic ganglia.

The second sort of tissue which takes part in forming some renal bodies is not so well known, and we shall use the name by which it has been most known, the *cortical tissue* of the renal bodies.

This tissue is composed, as a rule, of smaller cells arranged in cords which lie between a series of anastomosing blood vessels. The cells lie side by side in the cords, and form approximately rows which resemble true glands without a lumen. They probably secrete some substance into the blood.

These cortex cells are derived from some of the surrounding meso-

dermal tissue, and they, as well as the chromaffine cells, are sometimes found in widely different positions. The presence of bodies that correspond to renal bodies has been suspected in some invertebrates. This fact is far from being demonstrated, however, and the first of a very interesting taxonomic series of these tissues that we shall study are **the renal bodies of an elasmobranch fish**, *Raja maculata*. Both kinds of renal tissue, chromaffine and cortical, are found here, the former as a double row of bodies, one row on the ventral median edge of each kidney. They are larger toward the head, where they form the "axillary hearts." All the parts of these bodies are in intimate contact with branches of the large dorsal blood vessels.

A section of the anterior "axillary heart" shows that it is a body composed of both sympathetic nerve cells and the characteristic chromaffine

FIG. 282. — *A*, part of a section of a paired *adrenal* body from the skate, *Raja clavata*; *B*, part of a section of the *interrenal* body from the same fish. (After SWALE VINCENT.) × 450.

cells. These latter form the greater part of the posterior and internal part of the "gland." They are massed around a blood vessel as a zone of large irregular and branching cells which vary much in size. At the surface they show an irregular columnar arrangement due to their position. Figure 282, *A*, represents them taken in the center of the mass.

The posterior bodies of the two are much the same, except that the farther caudad they are found, the less becomes the nerve-cell portion. In the posterior third of the row the bodies are composed chiefly of chromaffine cells.

Besides these two rows of paraganglia there may be found another glandular mass without any duct between the two kidneys near their posterior end. Sections of this material (Fig. 282, *B*) show that it is made up of cortex cells which appear in cords and masses that lie in a sinusoidal plexus of blood vessels. The cords and masses are usually two cells deep, so that each cell has a proximal end in contact with the

blood supply, from which it is separated by a thin layer of tissue. Its distal end is in contact with the distal end of the cell opposite, and although there is no lumen visible, there is a tendency for the collection of secretion granules in the distal cytoplasm. In another fish, the conger, as figured by Vincent, a lumen does occupy this region and probably acts as a store for materials, as in the thyroid gland.

In higher forms we find the two tissues, just described in the skate, placed in varying degrees of proximity to each other. In the teleost fishes the cortical portion is large and separated from the very small amount of chromaffine tissue. In the Amphibia the two are in closer

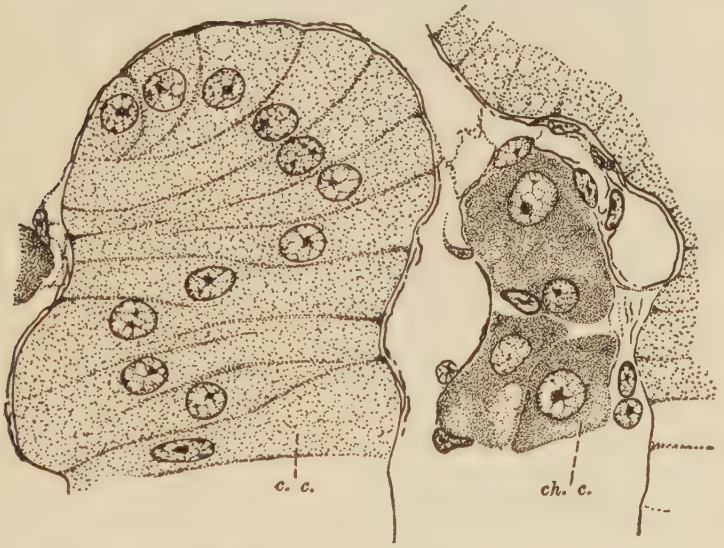

FIG. 283. — Portion of a section through the adrenal gland of a fowl, *Gallus domesticus*. *ch.c.*, chromaffine cells; *c.c.*, cortical cells. × 1000.

relation on the ventral surface of the kidney, while in the Sauropsida they are mingled closely in a single gland whose position much resembles that of the mammals.

Figure 283 shows a section of **the adrenal body of a bird**, *Gallus domesticus*, in which one can recognize the larger mass of cords composed of cortex cells, while among them at intervals appear smaller groups of chromaffine cells. The cortex cells show no lumen in their cords, although they are arranged so that every mass is double. Some cells are even placed in a cord, so that they do not have access to the blood supply. The body of such a cell must act in syncytial unison with that of the cells which do touch the blood vessel.

In the mammals the two adrenal tissues are placed close together, the

chromaffine cells forming a small inner medullary portion of the suprarenal body, while the cortex substance forms, as its name implies, an outer cortical part (Fig. 284). The cells of both cortex and medulla form irregular cords, and many sympathetic nerve cells are found at a hilum in contact with the medulla. The cords of cortex cells are extended radially from the contact with the medulla to the periphery of the organ. The blood supply is carried between the cords by wide, irregular capillaries or sinusoids. A more internal portion of the cortical cords is slightly different in arrangement, and forms an intermediate zone. The medulla varies in amount and does not extend into some of the smaller lobes, which are thus composed of cortex alone.

FIG. 284. — Section through cortex and a small part of medulla of the adrenal gland of a mole. *ch.c.*, chromaffine cells of medulla; *cor.*, cortex. × 500.

Another gland which is treated of here, because its function is not understood and because it has no duct, **is the coccygeal gland of man.** This gland may be dissected out as a closely associated series of larger and smaller masses of dense yellowish tissue, surrounding and adhering to the branches of the sacral artery.

A section of one of these masses (Fig. 285) shows that it is composed of several layers of the specific cells of the gland, adhering to the very thin walls of a wide blood space that receives blood from a small branch of the sacral artery. This blood space, or sinus, empties the blood through many fine vessels which pass distally through the gland-cell layers and collect the blood to deliver it into a neighboring vein.

The cells, as shown by their size, more compact and deeper staining cytoplasm, and their large nuclei, are evidently gland cells or secreting cells. They are not arranged so as to have any neighboring lumen and therefore must return their elaborated materials to the blood. Their portion with reference to the blood supply is accentuated by the very thin wall of the blood spaces or sinuses as well as by the fact that the blood

must pass slowly through such an enlarged vessel. The cells are thus in an advantageous position from which to receive food materials and

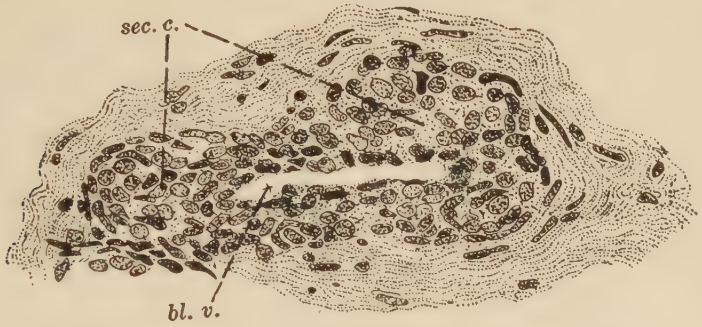

FIG. 285. — Section through a lobule of the coccygeal gland of man. *sec.c.*, secreting cells; *bl.v.*, blood vessel. (After WALKER.)

oxygen from the blood, to discharge their waste matter into it, and also to give up to it their secretion product, which must be of some use to the body or else the tissue would not exist. This gland is found in the body of man from fœtal life to death, and the one change which marks its greater age is a larger amount of connective tissue which does not exist at first in the cell masses.

On account of the anatomical position of the gland as well as the structure and physiological reactions of its specific cells, it has been supposed to be an homologue of the interrenal body of the elasmobranch fishes. This idea must remain as a mere speculation for the present.

Another gland of interest is **the carotid gland,** which is found in man as a small mass of yellowish red tissue placed at the bifurcation of the carotid artery. It is a little larger than a large grain of wheat, and might be compared closely to the coccygeal gland as to its structure. It has much the same kind of secreting cells, and these cells border upon a blood space from which they are separated by the very thinnest

FIG. 286. — Section of the carotid gland of man. *sec.c.*, secreting cells surrounded by blood channels. (After SCHAPER.)

of walls, a single layer of endothelial cells. A minor difference is that the cell mass is divided into smaller cords and plates which are in contact with a plexus of sinusoids instead of with a few larger sinuses (Fig. 286).

Technic. —The procedure for securing specimens of these tissues is simple, and demands but one detail, the use of some salt of chromic acid or of chromic acid itself in the fixative or hardening reagent. This insures the peculiar dark brown appearance of the chromaffine cells from which they have taken their name. The use of Flemming's fluid or of Zenker's fluid is thus indicated and gives the best results. Much of the work that has been done on these tissues has been done with Müller's fluid. Such work has been unsatisfactory in all details except as to the differentiation of the chromaffine cells.

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CHAPTER XVII

TISSUES OF RESPIRATION

The respiratory tissues form those organs by means of which an animal acquires its principal supply of oxygen, a gas that is absolutely needful in liberal and constant supply for the support of life. Unlike some food materials, oxygen cannot be stored for any length of time in the body, and therefore the organs of respiration are in constant use, even during sleep. In some forms sufficient air can be stored in these organs to last for a short time while the animal suspends breathing temporarily. Also, in other forms, an extremely slow form of respiration takes place during a state called hibernation.

The oxygen is always derived from the free supply of this gas in the atmosphere. When water is breathed, the oxygen is also obtained from the air that is dissolved in the water to which it has access, and not from the oxygen that constitutes a part of the water chemically. The respiratory tissues also serve as a medium through which carbon dioxide, a gas resulting from the use of the oxygen by the cells, is passed out of the body. The exchange of these two gases constitutes respiration.

The specific cell of respiration is a thin cell. Besides being thin in body, a feature of its cytoplasm is its clearness and non-staining property, especially of that part of it which is most directly used to transmit the gases. This probably comes from the absence of all materials or structures that might impede the passage of the oxygen and carbon dioxide. It is always placed between the air or water that supplies the oxygen and the tissue of the body that receives it. This receiving tissue is usually the blood.

The exchange of gases is probably not due to any specific physiological action of these cells, but rather to physical and chemical laws acting almost unrestrainedly through the body of the cell whose function seems to be one of self-elimination in the processes that are going on. This is not because protoplasm has not the power of handling gases physiologically and of operating with them against the activities of the ordinary physical and chemical laws (read next chapter, XVIII), but rather for the apparent reason that, since these necessary processes will go on by themselves, it would be a loss of energy to the organism to do it physiologically.

The respiratory epithelium is one of the most generalized tissues found in the animal body. On account of the negative nature of the duties to be performed, almost any epithelium can execute them, and in consequence, we find that the tissues devoted exclusively to respiration may be developed on a great variety of locations of the integument or on the inner surface regions of the body. Very many animals have no specific surface for respiration. Some of these, too, are otherwise highly organized, as, for instance, the earthworm, which utilizes its general body surface for that purpose. This use of the whole body surface might be looked upon as a specialization in itself.

The lack of cytological specialization also causes as great a diversity in the form of the respiratory organs as of their distribution. This diversity is well shown in the worms, for instance, where some of them have no special respiratory organ, while others have them in a variety of forms.

As a rule, animals breathing water use an evagination of some surface for a respiratory organ, while animals that derive their oxygen from the atmosphere use an invaginated surface for the same purpose. In the latter case the organ is designed to protect the delicate respiratory cells from the effects of drying. Cells, strong and resistant enough to stand drying, would not easily permit the gases to be exchanged and, besides, the moist condition is the most favorable for the process. Water-breathing animals may live in the atmosphere, and yet carry enough water in their body to use it for respiratory purposes, as do the land Crustacea and some fishes, while other organisms that breathe air may live a large part of their lives under water, and at the same time carry with them the air that they breathe.

Notwithstanding the negative character of a respiratory epithelium, it has, in many cases, retained some characteristics of the epithelium from which it has been derived. These characteristics are of no real use to it, usually, except the ciliated condition found in some forms. Here the cilia, which only appear on a part of the cells, are used to drive the currents of water over the respiratory surfaces, thus performing the act of breathing which in other organisms is performed by arrangements of cavities, valves, and muscles that belong to a morphological study of the subject. Some other residual characteristics found in the respiratory tissues are the chitinous covering found on the gills of the Crustacea, worms, etc., and the mucous cells and pigmented cells that can be seen on the gill surfaces in other forms.

The exchanges of gases do not take place through the respiratory cells alone. When the blood is held in a closed circuit of vessels, the walls of these vessels are also interposed between the blood and the respiratory medium. Thus the gases must pass through two layers of

tissue, the walls of the vessels as well as the respiratory cells. That either the walls of the respiratory epithelium or the walls of the blood vessels are not dispensed with, and one wall used to separate the two media, is possibly due to a number of reasons, among which can be brought to mind a lack of specialization in the coöperation of the two tissues. This may not have occurred because of the lack of any actual need of it or because of an inherent impossibility of an ectodermal epithelium becoming the wall of a blood channel, or of a connective tissue being situated on an external surface of the body. In some forms it is very hard or even impossible to detect the presence of this blood-channel wall.

The walls of the blood vessels, where they are in contact with a specialized respiratory epithelium, are as thin as possible, not more than one cell in thickness. This single layer is sometimes so thin that it can be seen with the greatest difficulty. In some cases it is clear that it does not exist, thus showing an exception.

The accessory tissues found in connection with the specific cells of respiration are but few in number. A small amount of connective tissue and muscle with a very small nerve supply are all that are directly concerned. The muscles, cartilages, and other tissues of the breathing passage and gills will not be considered here, as their functions are but indirectly related.

Technic. — These tissues may be treated as are any other delicate epithelia. When well hardened, they can be seen in section and distinguished from the underlying connective tissues by their hard outlines and compact texture, as well as by the peculiar transparent appearance that has already been commented upon. Teasing gives no important information, and is best put aside for the use of nitrate of silver on the fresh tissue. By staining the cement substances in this way, the boundaries of the cells are brought out and their relations with one another clearly demonstrated. Lungs should be gently distended as in life at the time of fixation, in order that the elements may present a natural appearance. Over distention is more harmful than the reverse.

LITERATURE

See the general text-books.

AIR-BREATHING RESPIRATORY TISSUES

The respiratory cells of the salamander are found on the inner surface of the lung, which is an invagination of the pharynx, and is provided with a supply of fresh air by the breathing of the animal. These cells line the entire inner surface of the lung and rest on a connective tissue with no well-defined basement membrane. Each one does not touch this

connective-tissue membrane at all points, but only with a comparatively small surface of its body, while the rest of its cytoplasm broadens out into a flange that reaches away from the pillar-like supporting mass and connects with the flanges of other cells. Thus an arch is formed between the two cells, and, as the cells are arranged in groups of three, all of whose points of contact with the membrane are contiguous, there is formed thereby a network of channels or spaces running everywhere between the outer cell-flanges and the connective tissue. These channels are occupied by the network of capillaries carrying the blood which is to exchange gases with the air-medium in the lung.

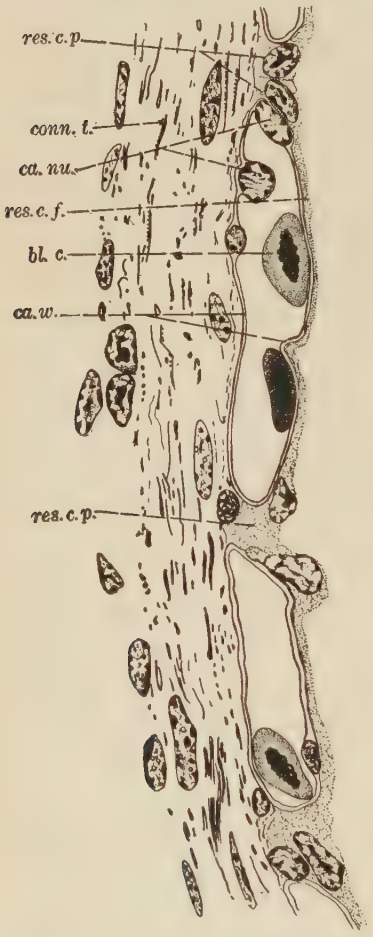

FIG. 287. — Part of a section of the wall of the lung of a salamander, *Necturus*. *conn. t.*, connective tissue; *res. c. f.*, flange of a respiratory cell (the functional part of the cell); *res. c. p.*, the pillars of two respiratory cells containing the nuclei and resting on the connective tissue; *ca. w.*, walls of the blood capillaries; *ca. nu.*, nuclei of capillaries; *bl. c.*, blood cells. × 435.

The capillaries have a single-layered wall of endothelial cells, and this wall is closely applied to the flanges of the epithelial cells distad, to the connective-tissue base proximad and to the attached bodies of the respiratory cells laterad on all sides.

In the section (Fig. 287) it can be seen that the large, round nuclei of the respiratory cells are nearly always in the supporting cell mass that rests on the connective tissue, while the elongated (disk-shaped in surface view) sections of the nuclei of the endothelial cells that form the walls of the blood capillaries are to be found at any point of the circumference of the section of the capillary.

This arrangement with modified detail holds for the vertebrate lung in general. Figure 288 shows a surface view of **the respiratory epithelium in man.**

Another form of air-breathing, respiratory tissue is found among certain mollusks. Mollusks are ordinarily water-breathers, and the water-breathing gill or ctenidium is a feature of much morphological importance. In a group

of the gasteropod mollusks, however, the ctenidium is not present, and another organ, an invaginated "lung" is formed in the mantle cavity to use air as a respiratory medium.

The inner surface of this lung is lined with the respiratory cells, which must be extraordinarily efficient if their structure can be taken as a criterion of their ability as a medium of gas exchange (Fig. 289). They are so flat and thin that they can with difficulty be distinguished, in section, from the cells they rest upon. The nucleus is of fair size, and is several times the general thickness of the cell body. At the point where it lies, the cytoplasm is thickened to contain it.

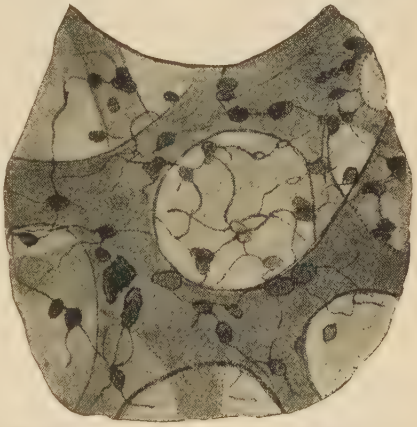

FIG. 288. — A surface view of some of the epithelium that lines the mammalian lung. Nitrate of silver preparation. (From Stohr's "Histology," after Lewis.)

In **the lung tissue of a wood snail**, *Triodopsis tridentata*, this flat epithelium covers the greater part of the cavity, especially such parts as are provided with the underlying blood channels. The cells bear no cilia, but in some parts of the cavity are portions of the epithelium that are composed of thicker cells, and these cells are provided with cilia. Such organs of motion are necessary in a cavity that is constantly invaded by particles of foreign substances carried with the air supply. They are so arranged that their concerted action passes the foreign

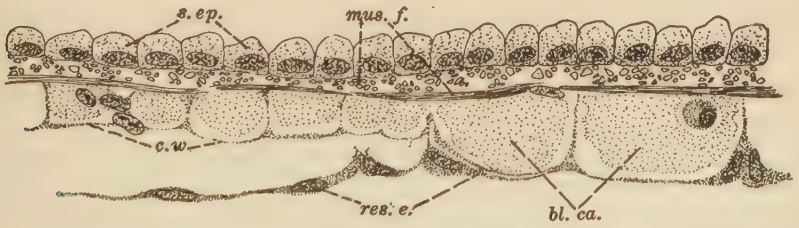

FIG. 289. — Vertical section of a part of the combined body wall and lung wall of a wood snail, *Triodopsis*. *s.ep.*, external shell epithelium; *mus.f.*, two layers (longitudinal and circular) of muscle fibers; *bl.ca.*, layer of blood capillaries, one of which contains a blood corpuscle; *c.w.*, single-layered capillary wall; *res.e.*, respiratory epithelium. It clings to the outer surfaces of the capillary walls in nature and is shown artificially separated in the left part of the figure. × 730.

particles along in the thin covering of mucus that covers them and expels the whole mass from the pulmonary opening.

The relations of the respiratory cells to the blood vessels on which

they rest is only clearly to be seen when a happy chance shows the epithelium torn partly away from the walls of these vessels. This condition is shown in Figure 289, and it can here be seen that the respiratory cells form a rather even layer, and do not have any portion of their cytoplasm extending down between the blood vessels to separate them and form "tunnels" for them, as was the case in the salamander. Some few processes of the cytoplasm do dip in far enough to secure an anchorage, but this is of small extent and occurs rather seldom.

The blood vessels with their thin walls form a very close and small-meshed plexus. On this account, and also because the vessels are large as compared with the meshes, all sections of them appear to be transverse sections or slightly oblique. The walls of the vessels are formed of a single layer of large thin cells, whose rather widely spaced nuclei appear but seldom in the section. Coagulated blood, however, containing a few typical mollusk blood corpuscles, fills the blood channels. Sections of the inter-vascular spaces or islands show merely a few connective-tissue cells.

The blood vessels rest on a longitudinal and a transverse layer of muscle fibers that lie between them and the shell epithelium of the outer integument of the animal. The blood must derive some oxygen through these latter thin outer layers.

Respiratory tissues that operate without the intermediate use of blood.—The above caption is not strictly true, as will be seen from the following account, but it will serve to materialize the principle involved. The tissue in consideration is the **tracheal respiratory system of the insects.** This structure consists, from an histological point of view, of an *invagination* of the same tissues that were *evaginated* in the lobster and other Crustacea to form gills.

These respiratory tubes, which have arisen by such invagination of the surface epithelium, branch and rebranch to ultimately form minute ramifications. It is the source of much controversy as to whether these ultimate branches anastomose or end blindly with a terminal cell. Be that as it may, this invaginated epithelium is carried as fine tubes to all parts of the body, and so generally distributed that all tissues can be supplied with oxygen directly from the tracheoles. Oxygen is thus distributed, and carbon dioxide collected without the direct intervention of the blood. In regions of great blood supply, however, the plexus of trachea becomes greatest, and by means of such plexuses the blood is most probably charged with a certain amount of oxygen for distribution.

All of these tracheæ are composed primarily of a layer of epithelium, which is derived from and continuous with the hypodermis of the body. The epithelium is composed of flattened, six-sided cells with large

disk-shaped nuclei (Fig. 290). They bear on their inner surfaces a layer of cuticle which they have elaborated. As we saw was the case in the water-breathing crustacean gill, this cuticle has undergone no further modification than to become as thin as possible. In the gill of the Crustacea the pressure of the blood was positive and inflated the evaginated structure. Here the pressure of the cœlomic fluids is negative, and tends to collapse the tube. In these invaginated tracheal tubes, therefore, the cuticle has been modified in an important manner. It has been thrown into thick, circular ridges, the *tænidia*. These serve to keep the delicate tubes open, and at the same time do not make the walls unnecessarily thick and heavy. Similar but not homologous formations are met with in the tube that leads to the large lung sacs of the mammals and other vertebrates. In this case there is also a negative pressure during expiration and cartilaginous rings are developed to keep it from collapsing. The conception of the trachea as invaginations of the outer surface is abundantly borne out by the embryological work on this structure. They can be seen in all stages of invagination. They do not, at first, contain air, this appearing at an early stage.

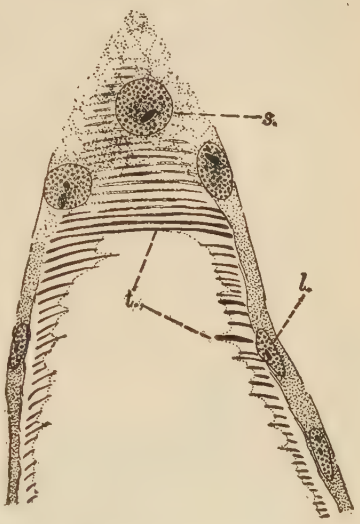

FIG. 290. — Part of an oblique section of an insect's tracheal tube. *s.*, surface view of one of the cells that make this tube; *l.*, lateral view of the same; *t.*, rings or tænidia of the cuticular tube. × 360.

Technic. — One must be somewhat more careful in fixing these tissues than in dealing with the water-breathing tissues. When once fixed and hardened the treatment is practically the same. When possible, the fixation should be done under a gentle pressure. The hardening as well as the fixation should be of some duration. Often it is of great advantage to fix by inflating the organs with the fumes of osmic acid and, after this has had plenty of time to act (long enough to "osmatise" the respiratory cells), the fixation can be finished by the use of any other fixative. Silver nitrate used on the fresh tissues may be made to show the cell outlines very beautifully.

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WATER-BREATHING RESPIRATORY TISSUES, GILLS

The water-breathing forms of respiratory tissues are the most primitive; at the same time their distribution is most diverse and their variation is greatest.

As an example of **a simple water-breathing, respiratory organ** we shall take the primary gill filaments of the embryo of *Acanthias vulgaris* (Fig. 291). In the embryo of most selachians the animal secures its oxygen and carbon dioxide exchange, with the embryonic fluids in which it lies, by means of a series of long filaments that grow out from the sides of the neck in the gill region.

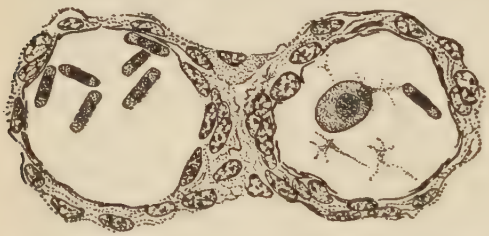

Fig. 291. — Transverse section of an embryonic respiratory filament of *Acanthias*. × 400.

Each filament consists of a long, single capillary loop embedded in a very small amount of connective tissue, and surrounded by an evaginated layer of the body epithelium. The blood passes down one side of the filament and returns on the other. The afferent and efferent capillaries that are seen in the section of the filament are lined with a single layer of endothelial cells and contain fully developed, red, nucleated corpuscles.

In the somewhat oval section of such a filament we find the respiratory cells to be a single layer of cells, a little too flat to be called cubical, and differing but little from the cells on the surface of the body from which they were derived, except that these latter are already stratified in a four-centimeter embryo into two layers or more.

The small amount of connective tissue that is seen, forms, for the most part, a septum separating the two vessels from one another. A few of these cells are to be found between the capillary and the respiratory epithelium but they are very much flattened. Undoubtedly parts of the cytoplasm of these cells separate the vessels from the epithelium at every point. The triple wall of epithelium, connective tissue, and endothelium is a fairly efficient organ for the transmission of gases when

the great length of the single filament is considered, and the fact that there is an exposure of the blood to the oxygen supply along its entire length.

A characteristic form of respiratory membrane is to be seen in **the gill of the lobster**. A long axis with nervous, muscular, and other structures bears a stream of blood out to its end and sends it off into a series of filaments. In cross section, such a filament is oval (Fig. 292), and its

FIG. 292. — Part of a transverse section of a respiratory filament of the lobster's gill. *bl.v.*, afferent and efferent blood vessels; *bl.ca.*, capillaries; *hy.*, hypodermal (epidermal) cells which line the body surface and produce the cuticle; *cu.*, cuticle; *ep.lam.*, thin layer of epidermal cytoplasm lying between a respiratory capillary and the cuticle; *conn.t.*, connective-tissue nuclei; *x*, unknown bodies near wall of one blood vessel. Arrows show how blood passes from artery (afferent blood vessel) to vein (efferent vessel). × 725.

outer edge is formed by a layer of covering cells in a loose mass of connective tissue containing the blood vessels of the filament. There is no basement membrane to separate the hypodermis from the connective tissue.

All blood spaces in the filaments appear to be channels lying among the Leidig's connective-tissue cells, and they are of two groups. First, there are the afferent artery and an efferent vein that together form a

loop to carry the blood into and out of the filament as was done in the dogfish embryo. Our figure shows one half of a section through a filament, and one of the large blood vessels, the artery.

But this vascular loop does not form a direct pathway. The second set of vessels are a set of fine capillaries that serve to carry the blood from the artery to the vein along their entire course. They leave the artery on its outer edge and extend around, as a plexus lying close to the surface, to empty into the outer side of the vein. They thus keep a large amount of the blood close to the surface, and in a suitable position for gas exchange or respiration to go on.

These capillaries appear to have no walls of their own, but to pass between the connective-tissue cells, between these and the hypodermis cells, or even between the hypodermis cells and the cuticle. They never quite reach this cuticle, however, as a small plate of cytoplasm belonging to the hypodermis cells always keeps them from directly touching it. This is well shown at *ep. lam.* in Figure 292.

The connective-tissue cells that form the central core of this filament are more characteristic than any others in the lobster's body. They do not show the periphery that the Leidig type of cell does, but have loosely branched protoplasmic processes. A very peculiar set of round objects which somewhat resemble nuclei are found on the inner side of the artery and are shown at *x* in Figure 292.

All the blood vessels, even the small capillaries, are lined with a very thin cuticular substance which gives them a clear and unmistakable outline that is well shown in the drawing. It should be remembered again that the surface of this gill consists of the same elements that the crustacean or insect body does, of an epithelium or hypodermis which secretes a cuticle, here modified by thinning for a special purpose. The organ is evaginated because it it to be used in water. If it were to be used in air, it would be invaginated as it is in the insects.

Note the thin layer of cytoplasm lying between the blood capillary and the cuticle at *ep. lam.* in Figure 292. This is not to keep the blood from touching the cuticle, but to provide the cuticle with a portion of cytoplasm which is the only agent which can make it and renew it when necessary.

Some of the gills found on worms are remarkable structures, and bear interesting histological relationships. The **gill filaments of the worm**, *Amphitrite ornata*, are good examples and a transverse section of one of these long, extensible filaments will show the desired features (Fig. 293). These filaments are used for other purposes than respiration, and it is not known whether the animal would perish at once, for lack of oxygen, without them. It probably would not, but would have time to regenerate them.

As can be seen, the filaments consist of a long core of connective

tissue containing two blood vessels that lie near opposite sides of the filaments. This core is composed of a very delicate connective tissue, and two bands of longitudinal muscle fibers lie on the edges farthest from the blood vessels.

The whole structure is covered with a tall, heavy, columnar epithelium whose cells show but poor lateral boundaries owing to the intimate way in which they are cemented together. A row of ciliated cells extends for the length of the filament on one side.

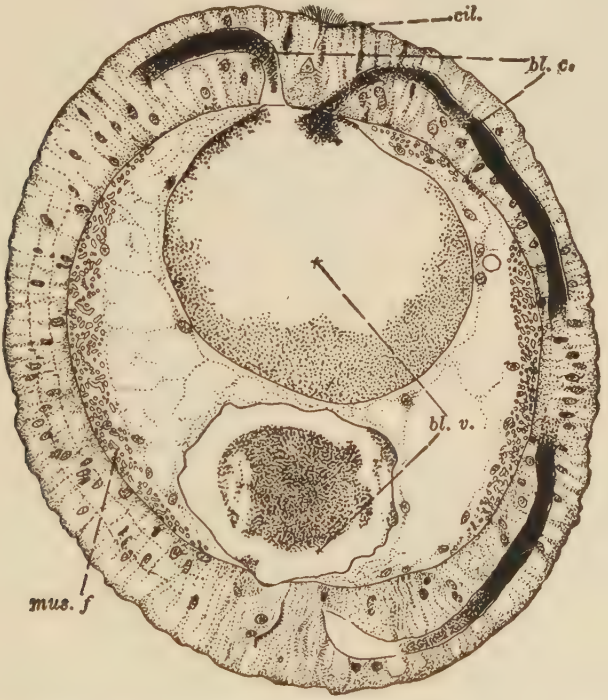

FIG. 293. — Transverse section of a respiratory filament (tentacle) of an annelid worm, *Amphitrite ornata*. *bl.v.*, blood vessels, smaller afferent vessel or artery, larger efferent vessel or vein; *bl.c.*, capillaries which conduct the blood from afferent vessel to efferent vessel through the respiratory epithelium; *mus.f.*, one of the two bands of longitudinal muscle fibers; *cil.*, ciliated cells on one edge of filament. × 400.

One of the blood vessels is large and the other smaller. This latter is probably an artery through which the blood runs faster, while it runs slower through the wider vein. The blood does not pass through the artery and vein as a simple loop. Instead it passes out of the artery, on its distal edge, into a great number of fine capillaries which pass both ways, *through the epithelium*, around to the distal edge of the vein, which they enter. The blood is thus brought into extensive and intimate contact with the outer surface, and is thus aërated and enabled

to throw off such impurities as it can in contact with the air (dissolved in water). No covering could be detected between the blood stream and the surrounding epithelial cells in which the vessel lay embedded. This form of tissue is much like that of the lobster.

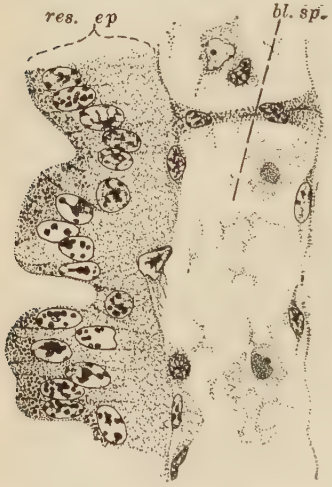

FIG. 294.— Central part and epithelium on one side of a gill plate of *Sycotypus*. *bl.sp.*, blood space containing a thin coagulum and a few blood cells; *res.ep.*, respiratory epithelium. × 800.

The mollusks that breathe water have a varied assortment of evaginated filaments, plates, etc., which are in some cases exceedingly complicated. We shall study the conditions first as shown in **the gill of the prosobranch gasteropod,** *Sycotypus* (Fig. 294). This gill is a double series of plate- or leaf-like evaginations, between whose double walls the blood slowly flows in very irregular capillary-like sinuses. These vessels are separated from the proximal edge of the epithelium by an abundance of loose connective tissue.

The epithelium is columnar and dense, and is as unspecialized a form of respiratory membrane as we have encountered. It is ciliated in many places and contains many mucous cells. The basement membrane on which the cells lie is well defined, but very thin in most places. On the two sides of the edge of each lamella it is thickened into two rods which are almost crescent-shaped in section. These are to be considered as skeletal structures used to stiffen the gill plate (Fig. 295).

The essential histological points of this respiratory tissue may be seen, accompanied by far more complicated anatomy, in the ctenidia of other mollusks. The squid and various lamellibranchs afford structures that will repay study by the exhibition of marvelous adaptations. Most of these other forms show a more specialized epithelium; one whose cells have given up other functions, and become as thin in body and as clear in cytoplasm as possible to permit of the ready passage of gases through their bodies.

The respiratory tissues of the fishes are found on evaginations and growths from the branchial arches. These take the form of a series of lamellæ which have arisen from the epithelium and its supporting connective tissue.

In the gill of the goldfish the general surface of the gill plate is covered by a stratified epithelium. The basal layer of this epithelium is

thrown into ridges which by continued evagination rise uncovered beyond the general surface of the stratified epithelium. These evaginations carry with them between their two walls a vascular plexus and a connective-tissue support (Fig. 296).

The blood vessels have a true capillary structure with definite endothelial walls of their own so that the contained blood is never in actual contact with the respiratory epithelium. These walls are thinnest where

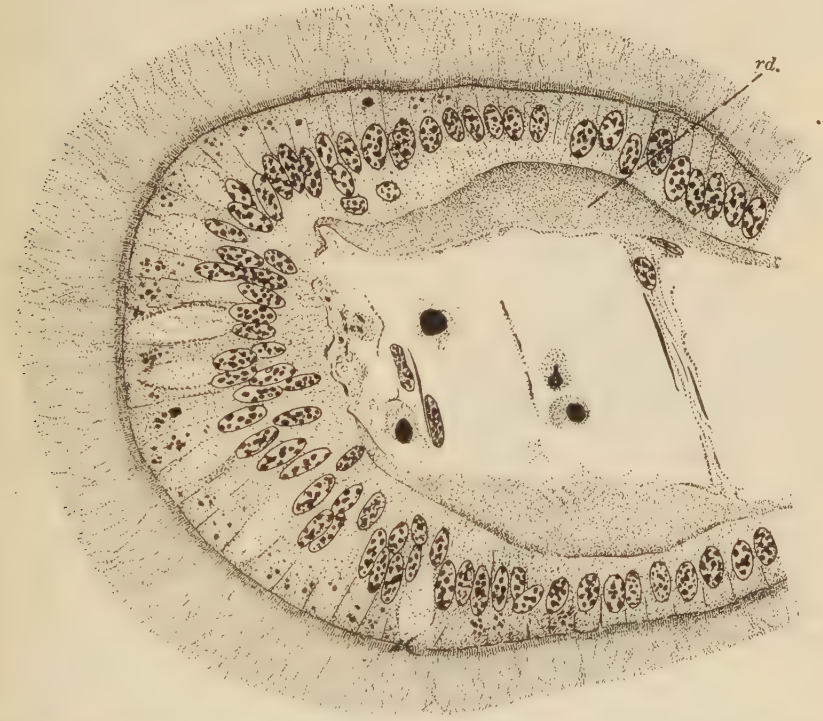

FIG. 295. — Transverse section of the edge of a respiratory plate of *Sycotypus*. *rd.*, one of the two chitinous rods which support the edge. The blood space shows blood cells and the epithelium is furnished with cilia. × 700.

they are next to the respiratory cells to permit of the freest gas exchange. They are decidedly thicker and heavier where they lie in the meshes of the plexus or between the capillaries as they appear in the figure. This is probably to afford support for the lamella. The epithelium as a whole must be carefully studied. Where it remains on the surface of the gill bar and between the lamellæ it is typically stratified, the basal layer proliferating freely and the proliferated layers lying in a mass that reaches halfway up between the lamellæ. The cells of the uppermost or superficial layer of this mass are enlarged, and dense with the nucleus

situated proximally in their bases (Fig. 296, *ep.c.*). One or two of such cells will be found between most lamellæ. Any particular function which they may possess is unknown.

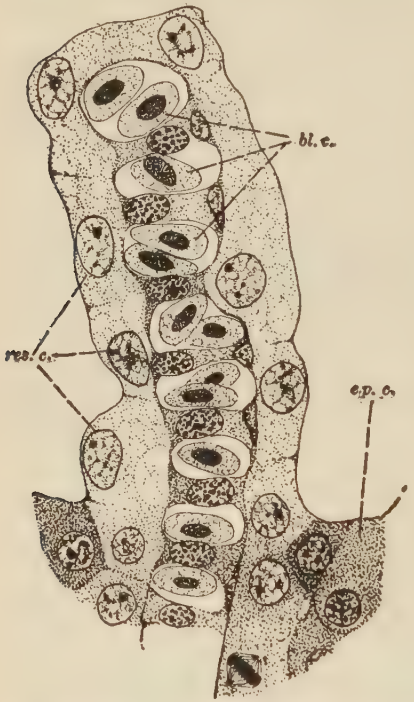

FIG. 296. — Part of a section through the gill filament of a goldfish. *res.c.*, respiratory cells; *bl.c.*, blood cells in the many sections of capillaries; *ep.c.*, unmodified outer cells of the stratified epithelium from which the gill is developed (possibly has some obscure glandular function). × 1300.

Those cells of the basal layer which are reflected over the lamellæ become the true respiratory cells (Fig. 296). In this position they have relinquished the work of desquamation, and consequently remain a simple epithelium. Their renewal, when injured or worn out, is secured by mitotic cell-division in the fundus and on the lower sides of the lamellæ, and the moving of the whole layer up to fill the gap. A mitotic figure which is probably fulfilling this duty is shown on the middle side of the lamella in the figure.

In structure the respiratory cells are decidedly specialized. They do not become so flat and thin as many other cells do under the circumstances, but they broaden and become lighter, and the cytoplasm becomes far less dense than it is in the lower parts.

Technic. — The gill tissues are very easy to cut in paraffin on account of the delicacy of the various structures which have to be so because of the necessity of allowing the oxygen to pass through. For the same reason the organs are quite hard to fix without shrinkage and distortion. Flemming's fluid, Zenker's fluid, and chrom-aceto-formaldehyde gave good results, especially when allowed to act for a long while.

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CHAPTER XVIII

THE GAS-SECRETING TISSUES OF ANIMALS

Among the many substances that cells can produce by the process of secretion is free gas. Of course we have seen in the preceding part that very many tissues can allow gas to be transmitted through their substance under physical and chemical impulse (pressure and chemical affinity). But the cells to be discussed in this part are able to take the gas materials from the blood and to secrete and discharge them into a chamber that is under a pressure greater than that of the surrounding medium in which the animal is placed. As several gases are so handled as a mixture, there are many unknown chemical changes involved. The gas appears as tiny solid granules in the cytoplasm of the cell and swells into a fluid droplet, and then into the gaseous state, when it is discharged from the cell.

We shall examine two of the very few forms of animals in which this occurs, a teleost fish whose swim-bladder is filled with a mixture of gases and a siphonophore medusa, one of whose zooids is developed into a hollow float that is also filled with about the same mixture of gases that we found in the swim-bladder of the fish. This mixture is CO_2 in 2–6%; Oxygen, 12–18%; Nitrogen 79–80%.

Gas secretion in the siphonophore medusa, *Physalia*, and others. — The float member, of the collection of individuals that a *Physalia* represents, is developed by an invagination, into a large hollow, double-walled sac or bladder with a pore at one point that controls the exit, and the consequent pressure of the gases by a sphincter muscle. When gas is lost, by letting it out or when the supply is decreased by the growth and enlargement of the float or by the loss of gas by osmosis through the walls, a new supply is provided by a portion of the epithelium that is situated on the inner membrane near the base of the float (Fig. 297).

This epithelium, which lines the membrane and faces the hollow of the float, consists of a single layer of long cells with a swollen distal portion that narrows down into a rod-like base near the basement membrane, where it branches into several root-like processes that are implanted in the jelly tissue of the mesoglœa. The nucleus is of large size and placed

where the basal portion of the cell begins to widen, about one third of the distance from the base. The secretion first appears as a group of small granules immediately distad of the nucleus, and these granules move toward the distal end of the cell, where they swell and become filled with the gas. The large gas bubbles rupture the cell-wall and break into the gas chamber to supply it. The whole membrane with its epithelium is thrown into a series of parallel folds of moderate depth. These folds become of lesser depth on either side of a central area.

FIG. 297. — Two gas-secreting cells from the gas epithelium of the siphonophore medusa, *Physalia*. *v.*, small vacuoles; *chr.v.*, the chromatic vacuoles: *b.*, bundles of mesodermal tissue belonging to gas epithelium; *b.*, smaller mesodermal bundles belong to the corresponding endodermal epithelium, the bases of whose cells are indicated by lines. × 1000.

A short account of the **gas cells of another siphonophore**, *Physophora hydrostatica*, which occur in a more highly specialized organ, should be considered here.

The gas cells in this form are found on a membrane homologous to that which bears them in *Physalia*. But all of the epithelial cells on this membrane are not developed into the gas cells. Most of them are a simple cell representing the secondary ectoderm (from which the gas cells also arise) in its simplest form. Only, instead of both sorts of cells forming a single row, the undifferentiated cells form a thick, many-layered mass in which the gas cells are placed sparingly and always away from contact with both basement membrane and distal surface.

These cells secrete the gas in much the same way that it is done in *Physalia* except that the vacuoles of gas must force their way to the surface and break out into the gas chamber.

An even more **highly specialized form of gas cell is found in a third form of siphonophore**, *Rhizophyza filiformis*, which occurs in the Mediterranean Sea (Fig. 298). This large gas cell has a huge nucleus of peculiar texture, which is shaped like a kidney. On the hollowed side

of this nucleus is a sphere of darker staining cytoplasm that appears much like the centrosphere of some spermatogonia. Around this sphere is a zone of lighter staining substance that is yet darker than the cytoplasm, and whose ends are expanded on the side farthest from the nucleus into two wing-like processes that reach almost to the cell-wall. The secretion appears in the form of fine granules that swell and finally are transformed into the gas near the periphery of the cell.

FIG. 298. — Part of a gas-secreting cell from the siphonophore medusa, *Rhizophyza filiformis*. *nu.*, nucleus showing a dark and crowded chromatin pattern; *x*, unknown centrosphere-like body. (After K. C. SCHNEIDER.)

Gas secretion in the teleost fish, *Gadus morhua*, and others. —Many of the teleost fishes possess a swim-bladder that serves to reduce their specific gravity by secreting and containing a gas mixture. The gas is secreted by the epithelial lining of the organ.

As the swim-bladder is formed by an embryonic invagination of the intestinal tract, this gas epithelium is genetically related to the respiratory cells of the vertebrate lung, whether the swim-bladder and the lung are the same or different invaginations, phylogenetically, of this region or not.

But while the respiratory cells passively allowed various gases to diffuse themselves through their cytoplasm, this epithelium of the swim-bladder, as has been said, secretes it into a chamber that is under a mechanical pressure, and perhaps a chemical condition of resistance as well.

The epithelium sometimes covers the entire inner surface of the swim-bladder, while in our subject, the cod, only that portion on a limited area of this lining epithelium is so used. This smaller part, however, is specialized into a condition of greater efficiency by, first, an amplification of its surface through numerous tubular and folding invaginations and, secondly, by an increase in the size and thickness of the secreting cells themselves (Fig. 299). This is accompanied by an increased peripheral blood supply that is pushed up in capillary loops into the regions between

the invaginations. A peculiarity of this blood supply is the way that the blood stream is divided. The dendritic method of division found in the circulatory supply of most organs and tissues, where the artery divides into branches and subdivides until the stream is running in capillaries, is replaced by a great mass of capillaries that arise together on a few large vessels that are found on the inner surface of the bladder and run in a mass with parallel courses to the secreting epithelium. Here they enter, and having entered, they begin to separate from one another. Toward the outer surface of the mucosa, as we shall call the layer of invaginated epithelium together with the connective and other tissues that are involved with it, the capillaries anastomose and become slightly larger. They may be designated sinusoids here. So complicated have the histological relations become in this mucosa that the epithelial nature of the secreting cells is doubtful without careful study. Many inner cells are apparently devoid of a disto-proximal differentiation, owing to the fact that the lumen of the gland is closed by the absence of any secretion at the point where they are. In places where the comparatively scarce lumina of secreting acini push their way down into the mass, the relations of the cells are clearly seen, and in some cases they appear typically columnar, resting on the blood channels from which they draw their materials with the even surface of their ends bounding the round, open lumen. Such a lumen was probably full of gas at the time of fixation.

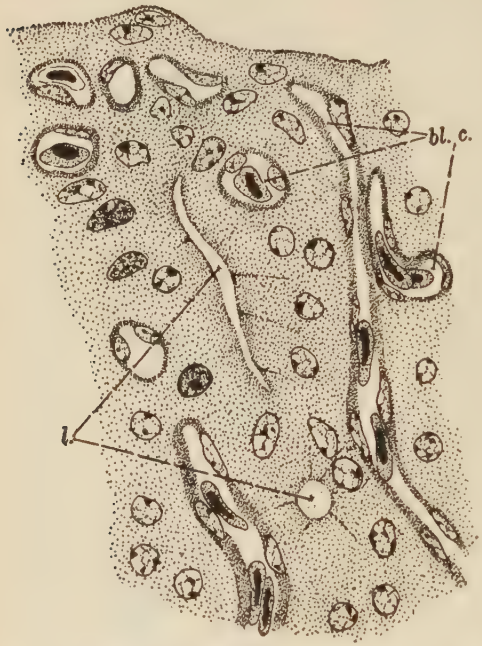

FIG. 299. — Part of the distal region of the gas-secreting gland of the cod, *Gadus*. *bl c.*, blood capillaries with endothelial walls on which the proximal surfaces of the gas cells rest; *l.*, parts of two lumina on which the distal ends of the gas cells border. × 1000.

The epithelium directly on the primary surface of the gas gland is slightly different from the cells bordering on the secondary lumina, and probably have in addition to their duty of gas production the work of secreting the peculiar layer that lines the entire inner surface of the swim-bladder. The gas bubbles slowly force their way through this layer,

which parts before the pressure and closes again when the bubble has passed.

The cytology of the typical gas cells is peculiar. As this is more easily seen in those of some other fish than the cod, we shall examine them in **the swim-bladder of the golden paradise fish**, or so-called Japanese goldfish (Fig. 300). This tissue has been described by Reis and Nusbaum.

Fig. 300. — Five gas-secreting cells from the gas gland in the swim-bladder of the paradise fish, *Macropodus viridi-auratus*. *b.*, thickened distal border of the cells on the lumen; *vac.*, gas-vacuoles; *tr.*, trophospongia; *bl.ca.*, capillary. (After Reis and Nusbaum.)

In this form the differentiation of a distinct gland is but partial, the thick heavy gas cells lining the entire interior of the swim-bladder, and being invaginated to some degree on an area of the ventral surface only. Here the few tubular invaginations extend down into the connective tissue lying between the epithelium and the wall of the swim-bladder.

Cells taken from any part of the gas epithelium will answer. Such a cell is cylindrical with its base resting on a blood channel and its distal end touching the lumen of the bladder or one of its branches in the lumina. Its base is somewhat thickened and stains deeper owing to materials placed here that do not occur elsewhere. These materials, which at best can be only identified as fine granules amid a network of irregular fibrils, are either some particular organ of the cytoplasm that is used to collect the gas-forming materials from the blood, or they represent those collected materials themselves. A combination of both conditions is the best explanation of this appearance.

These materials must certainly be passed forward through the cytoplasm of the cell as fluids or as very fine granules. This can safely be assumed, yet no trace of their presence is visible until we have gone distad of the nucleus in our search, and come to the outer third of the cell. Here the materials for the making of the gas are once more to be seen, collected as granules in an important organ of the cell, a series of fine cytoplasmic channels that gather at their center into a central cleft of irregular outline. The lumen of this channel system is not free but filled with another and more fluid form of cytoplasm. The cytoplasm of this region elaborates the materials into the gas or into granules of substances that readily combine to form the gas, and these granules are passed down the channels to the cleft. In this space they are converted into the gas mixture which

appears as a number of tiny bubbles that unite to form larger vesicles. These latter work to the surface of the gland or inner surface of the swim-bladder and are discharged into it.

When the gas is formed, there is a residual material that remains as a mass of solid granules that are discharged with or after the gas. This is a by-product of the chemical processes by which the gas was formed.

The entire mucosa in the cod is divided into a large number of lobules that take an independent origin from the basal tissues. Between these lobules the epithelium with its underlying connective tissue is evaginated into a series of folds that rise above and cover over with their edges the lobule, forming in this way a common covering for the entire organ, but leaving openings through which the gas may escape into the bladder.

This covering is thus lined on both its upper and its lower surfaces with the undifferentiated lining epithelium of the swim-bladder, and the central layer is composed of a connective tissue of fine texture in which run arteries and veins. The meaning of the structure is not plain, and requires further study. It allows the gas to escape from the gland by the parting of the sticky edges of its several parts, which then drop back into place.

Technic. —There are no special methods which have been evolved for the purpose of bringing out any of the specific features of the gas epithelium of the gas gland. Flemming's fluid and Zenker's fluid serve to fix the tissues so that all the known structures may be seen when the sections have been stained in iron hæmatoxylin.

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CHAPTER XIX

THE EXCRETORY OR NEPHRIDIAL TISSUES

The life of all animals depends upon a double process by which complex tissue substances are built up to be broken down later in the release of some kind of energy. This double process of building up and breaking down is known as *metabolism*. It involves the securing and distribution of food and the collection and elimination of waste products resulting from the breaking down of tissue materials. Tissues of alimentation and circulation take care of the securing and distributing of the food. The nitrogenous waste products of metabolism are poisonous to the tissues not differentiated for their reception, and must be readily removed from the animal body or be stored in some tissue highly modified for their reception. Tissues of urine excretion, therefore, have evolved along with the advance in animal structure. In all cases, except in the ascidians, these tissues are organized in such a manner that the nitrogenous waste products can get out from the body. These structures vary much in their complexity.

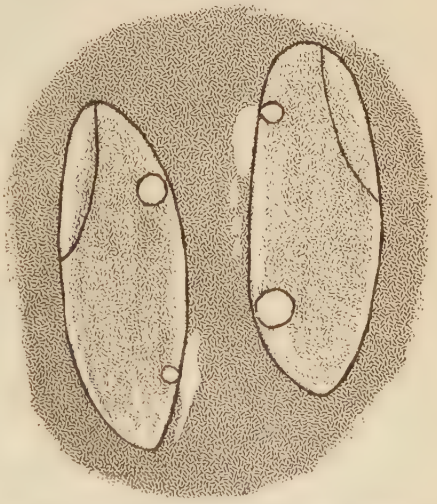

Fig. 301. — Two infusorians swimming in a solution of India ink. The matter discharged from the pulsating vacuoles may be temporarily seen as an irregular area of clear fluid next to the body and surrounded by the darkened water. (After Jennings.)

Among the unicellular forms we have seen that the alimentation and distribution of food was effected by means of vacuoles within the cytoplasm of the cell. So in the same simple forms we have vacuoles forming channels leading from the endoplasm to the exterior of the cell body. Because of their power of rhythmic contraction they are called *contracting vacuoles*. Figure 301 shows that these vacuoles expel their fluid contents. The fluid that the vacuoles constantly throw out in this

manner is drained from the cell. Griffiths, in 1889, showed that this fluid is charged with uric acid. This type of excretory organ is common to all unicellular organisms in which there is a comparatively great activity. In other words, where much energy is displayed, many waste products are formed, and an excretory cell structure has arisen to handle them. Where the activity carried on by the unicellular form is low there seems to be little or no necessity for such structure, the general surface of the cell serving as a medium for the discharge of the waste products. Such a contrast is seen in the gametes of certain Algæ. The active male gamete has one or two contractile vacuoles and the passive female gamete has none.

An example of contractile vacuoles. — The contractile vacuoles of *Paramœcium aurelia* or *Paramœcium caudatum* are always two in number. Unlike food vacuoles, their number is constant, and they are stationary; also, they are permanent features of the cell. Their inner

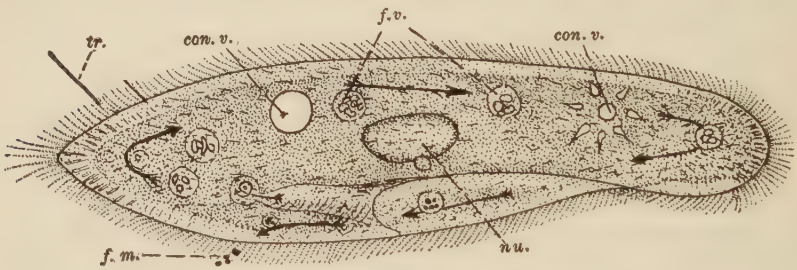

FIG. 302. — Individual of *Paramœcium caudatum*. Arrows show course of food vacuoles (*f.v.*). *nu.*, nuclei; *con.v.*, contracting vacuoles, one empty and one full; *f.m.*, fecal matter; *tr.*, discharged trichocyst. × 375.

surface dips into the endoplasm and their outer surface opens through the ectoplasm to the exterior. Into each contractile vacuole a radiating series of drainage channels lead (Fig. 302). These channels empty their contents into the contractile vacuole. The channels are filled as the contractile vacuole discharges its contents. These vessels are best seen when filled. So the channels become conspicuous as the vacuole becomes indistinct.

In the cœlenterates there are two factors which in part account for the absence of specialized excretory structures. First, the activity of these forms is low, resulting in the formation of a relatively small amount of waste products. And secondly, all of these cells except the very passive mesodermal cells have a surface exposure. It is quite probable, therefore, that the surface exposure in the cœlenterate body is sufficient to remove the relatively small amount of urine evolved.

Waste products of the internal cells are thrown off by these cells into the intercellular mesenchymal spaces to be passed to the epithelia

covering the surfaces of the body. Arriving at the surface in a medusa, for example, these materials are cast out into the surrounding water through the layer of epithelial cells which cover the surfaces. In this connection we must bear in mind that the intercellular fluid carries food materials as well as waste materials. These and other valuable materials are not taken up by the epithelial cells to be thrown out from the body. We see, then, that these cells can discriminate and select only the materials which must be removed from the internal tissues and fluid; with the possession of this power they become *excretory* or *nephridial cells*. These comparatively simple forms of excretory cells have not been differentiated into a distinct excretory tissue; they perform other functions as well as that of excretion.

In the more active Metazoa, where a greater differentiation has taken place, tissues have been specialized that perform only the function of taking up waste substances from collecting fluids. These tissues have the power to take enough food from the body fluids for their own nourishment. Except in disease they take no more of the food materials than this. On the other hand, they select most of the waste products from the collecting media and transfer them to the exterior. This transference is a vital and not a mechanical process. In this respect, it differs from the transfer or exchange of gases in respiratory tissues.

Tissues specialized for the selection of urates from collecting and distributing fluids form the *nephridia*. In the higher vertebrates, the nephridial tissues are assembled and, together with their special blood and nerve supply and connective-tissue elements, form an excretory organ, the *kidney*.

The collecting fluids are *intercellular fluid*, *cœlomic fluid*, and *blood*. Intercellular fluid and cœlomic fluid when associated with nephridia bathe them on their proximal surfaces. The blood supply is effected in two ways. In a few types of animals the nephridial tissues are merely bathed in the blood. The Insecta furnish a good example of this. This first mode of blood supply for nephridial tissues is very unusual. Blood is usually supplied to the nephridial tissues through the capillaries of a circulatory system. In the simplest tissues there is but an ordinary capillary supply. This becomes more highly specialized in the higher forms. In the vertebrates there is a general capillary supply as well as a terminal supply. The terminal capillary structure is a more or less distorted *plexus* which is supported upon a connective-tissue framework at definite terminal regions of the nephridial tissues. This latter capillary structure is called a *glomus*.

The nephridial tissues may have various origins. We have seen that in the lowest Metazoa, so far as known, any surface cells may perform nephridial functions. The higher forms of nephridial tissues are

usually mesodermal structures. They all (except the ascidians) have effected a subsequent and secondary relation to the ectoderm.

These tissues are always epithelial. One face, the proximal surface, of an excretory epithelium is directed toward the fluids from which waste products are being taken; the other face forms the surface of a retaining or conducting cavity. These tissues form, therefore, sac-like or tubular organs. In the ascidians the renal epithelium is a vestigial cœlomic epithelium. Into this blind space waste products are excreted and stored as solid particles. All other nephridial sacs or tubules deliver the waste products to the exterior through nephridial pores and ducts. In all the simpler forms where the nephridial tubules have a small lumen, the latter is intracellular. In invertebrates where the lumen becomes large, and in all vertebrates, it is intercellular.

The character of the fluids with which nephridial tubules are functionally associated, and the manner in which blood is brought to them, has much to do with their structure. Tubules associated with intercellular fluid, simple lacunar blood supply, or with certain cœlomic fluids, have usually two distinct regions. These two regions are constituted by a system of excretory tubules and by terminal excretory cells, of a peculiar type. In these forms the lumen is usually intracellular. In the flat-worms we have such a nephridial system associated with a simple collecting medium. In the nemerteans the nephridia, though associated with a circulating blood, are fundamentally like those of the flat-worms In the rotifers similar nephridia are associated with a cœlomic fluid. On the other hand, there are no specialized end cells in the simple nephridia of the nematods. This latter may represent a case of retrogression. We shall later examine, as an example of these simple nephridia associated with the simpler collecting fluids, the nephridia of the tapeworm commonly found in the intestine of the robin.

Tubules bathed in a blood supply are blind and have a uniform structure throughout their extent. We shall take as an example of this type of nephridia the tubule of the insect.

Certain forms in which the nephridia have a cœlomic fluid from which to take excretory fluids and a general distribution of capillaries over their walls have uniform structure throughout their extent and end blindly. In connection with the work they have to do on the cœlomic fluid their blind ends bear a group of peculiar terminal cells similar to the terminal cells that in lower animals act upon cœlomic and other simple collecting fluids. Fage, Goodrich, and others described such nephridia in various polychætes. We shall take *Eulalia viridis* Müll. for an example.

Certain tubules with a general capillary supply, and having no particular direct relation to the cœlomic fluid, are simple and undifferen-

tiated throughout their course. Such an example we have in the mollusk, *Sycotypus*.

Tubules associated with a more complex supply tend to become differentiated into distinct regions. In the highest forms where the capillaries form terminal *glomi*, we have developed the tubule characteristic of all vertebrates. We shall take as examples the various complex nephridial tubules of *Lumbricus*, *Homarus*, and *Iguana*.

In certain vertebrates a group of tubules have in common one *glomus*. In these cases the tubules end with more or less expanded walls which open into the cœlom. These open, expanded ends always have strong cilia which are directed from the cœlom toward and into the lumen of the tubule. Such a terminal structure is known as a *nephrostome* and it may remove solid as well as fluid substances from the cœlomic fluid. Among the invertebrates we shall take as examples of nephrostomes of simple, intermediate, and complex structure those of *Polygordias neapolitanus*, *Perichæta malamaniensis* Benh., and *Lumbricus herculeus*, respectively. The nephrostome of *Ammocötes* will be taken as an example of the vertebrate nephrostome.

In connection with the nephrostomes, especially among the invertebrates, interesting accessory structures are developed. The general cœlomic epithelium may be considered in some forms as excretory in function. Such is clearly the case, according to Schaeppe ('93), in the cœlom of the annelid, *Ophelia*. The peritoneum of this worm elaborates chloragogen in the form of granules of *guanin*. In the earthworm the peritoneal epithelium gives off cells which become amœboid and have the power to elaborate excretion granules. Among certain mollusks the pericardial epithelium, which is a cœlomic structure, becomes intensified by the formation of glands known as the *pericardial glands*. These give off wandering cells or *amœbocytes*, which, after they have elaborated their concretions, find their way out through a canal known as the reno-pericardial canal. This latter canal, therefore, is functionally, if not morphologically, a nephrostome. As examples of such accessory renal structures we shall take the cœlomic epithelium and the wandering cells of *Lumbricus* and the wandering cells of the pericardial gland of *Unio*.

The function of highly modified cœlomic cells called *chloragogenic cells* which are found investing parts of the alimentary canal of many invertebrates is yet to be satisfactorily demonstrated. According to Ladreyt ('04) these cells in *Sipunculus modus* Linn. excrete uric acid. For convenience they will be described in this connection. As an example we shall take those found about the intestine and large blood vessels of *Lumbricus*.

Also as a matter of convenience we shall here describe as a type of

excretory cells the calcium phosphate cells which Barfürth so names and describes for *Helix*. We shall take as our example those found in the hepato-pancreatic epithelium of *Mesadon*. It is interesting to note in this connection that the concretion particles found in the renal sacs of *Nautilus* contain, according to Keferstein, calcium phosphate bodies and other salts, but no urine.

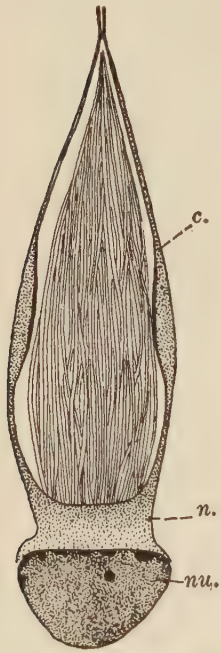

FIG. 303. — A flame-cell from the tapeworm found in the robin. *nu.*, nucleus; *c.*, collar; *n.*, neck. Mass of cilia inside.

In the vertebrates and some invertebrates, well-developed conducting channels and retaining vessels for urine are developed. These are the *ureter*, *bladder*, and *urethra*.

The sweat-glands elaborate a certain amount of urine and other waste products. These structures have been considered in connection with the integument. It is of interest to point out at this place that they too are epithelial in character.

Nephridial tubules. The nephridia of the tapeworm of the robin. — The mesenchyme of a tapeworm contains many intercellular spaces filled with a body fluid. Into these passages waste solutions are emptied. This waste is taken from the body fluid by numerous excretory cells which deliver the collected material to a system of excretory capillaries. The walls of the capillaries are composed of very thin tile- or plate-like mesenchymal cells. The ultimate branches of these capillaries bear the excretory cells. These cells have a cytoplasmic body which develops a collar (Fig. 303). The collar forms the terminal part of the wall of the excretory capillary. Into the lumen of this collar the terminal cells send a tuft of heavy cilia which in life flickers in a manner that has suggested the term "flame" for it. A large rounded nucleus lies in the distal part of the cytoplasm (Fig. 303, *nu.*). This type of cell is known as a "*flame-cell*" or *solenocyte*.

The nephridium common to insects is a blind tubule known as a *Malpighian tubule*. The Malpighian tubule of a caddis-fly larva is simple and unbranched. The wall is composed of two rows of grooved cells that have almost lost their individuality to form a syncytium. The nuclei on one side of the tubule alternate with those on the other. They are large, and have their chromatin uniformly distributed as spherical granules. The cytoplasm is rather dense and homogeneous. The inner margin bears a less opaque striated cuticula. The lumen is rela-

tively small. In it excretion products are seen. The tubule is incased in a *membrana propria* or basement membrane (Fig. 304). This gland is a modified and invaginated portion of the intestinal epithelium.

FIG. 304. — Transverse section of a renal tubule of a caddis larva. The tubule is formed (in transverse section) of two cells which, because of the alternation of their central masses, show but one nucleus in any given cross section. The distal surfaces of the cells, where they border on the lumen, show a cuticular edge. × 550.

Eulalia viridis Müll., according to Fage's description, possesses **nephridia which are more complex than those of the flat-worm.** The nephridium has a conducting tubule. The lumen of this tubule is intracellular, and its wall is a syncytium. These latter features are frequently met with in excretion tissues. There are a few scattered cilia in the lumen. The nuclei are not frequent. The inner zone of cytoplasm is extremely finely granular. The outer zone is marked by striæ which stain deeply. This nephridial tubule bears distally a row of solenocytes. Each solenocyte is represented by a mass of cytoplasm which is fused with the cytoplasm of its neighbor. The nucleus lies in this cytoplasmic body. Each cytoplasmic mass gives off a collar which pierces the wall of the tubule to empty into its lumen. The wall of this collar is highly modified and in being less soluble in caustic potash than cytoplasm shows a marked differentiation. Its length is twenty to twenty-five microns. It has a very small lumen. Within this lumen Fage describes a single, slender flagellum which may lie beyond the collar into the lumen of the nephridial tubule. A supporting membrane is found rising from the nephridial tubule and giving off external flagella which probably

FIG. 305. — Inner end of a renal tubule of the worm, *Eulalia*. (After FAGE.)

direct currents of the cœlomic fluid toward the solenocytes (Fig. 305); such solenocytes with long and powerful cilia have also been described by Goodrich ('00) for *Asterope candida* Cam. The solenocytes probably take up waste materials from the cœlomic fluids. The wall of the nephridial tubule has a blood capillary supply. In the worms the chief amount of excretion products is taken from blood by the nephridial walls. This rather complex type of nephridium in modified forms seems to be present in many polychætes.

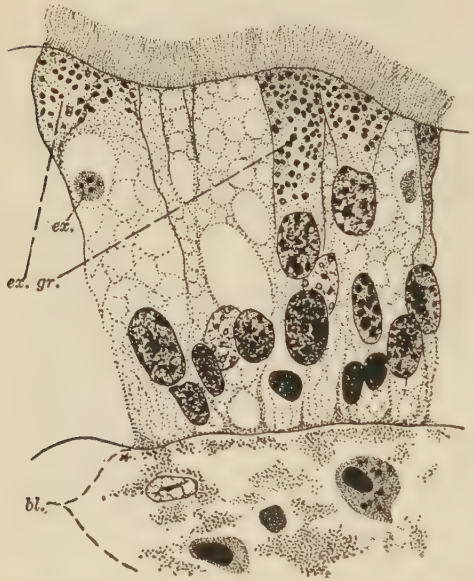

FIG. 306. — Bit of renal epithelium from the gasteropod mollusk *Sycotypus*. *bl.*, a blood sinus containing blood cells and coagulated blood; *ex.*, large excretion particle in cell; *ex.gr.*, a second kind of excretion substance in other cells. × 1000.

The nephridium of *Sycotypus* is a greatly contorted sac. The wall is furnished with a connective-tissue coat which bears many blood vessels. These vessels open into sinuses along the basement membrane of the nephridial epithelium. Figure 306 shows coagulated blood serum containing blood lying next to the basement membrane. One corpuscle bears granules which resemble the excretion granules found in certain of the epithelial cells of the renal sac.

The epithelium forms no varied regions in the sac. It is composed of tall, columnar cells measuring in height about forty-five or fifty microns. These cells are all ciliated. Oval nuclei lie usually in the basal third of the cell. This is not a constant position for the nuclei. In some cases nuclei may appear quite near the distal ends of the cells. The various nuclei do not stain uniformly in iron hæmatoxylin. The cytoplasm is alveolar to highly vacuolated. The cells present two conditions. In one condition the cell body is rounded, tending to be cylindrical. The cytoplasm here is greatly vacuolated, and contains one or two rather large excretion bodies or bears no excretion particles. In the other condition the cells become greatly flattened so that they become somewhat fan-shaped. These bear numerous refractive, angular excretion particles (Fig. 306, *ex. gr.*).

The nephridial tubule of *Lumbricus*. — This tubule is differentiated,

according to Benham, into five regions: (1) the narrow preseptal tube; (2) "the very long but narrow tube in continuity with the preseptal tube"; (3) "the short, brownish, ciliated, middle tube"; (4) "the wide muscular tube or duct which opens to the exterior." Each of these except the preseptal tubule and the short, brownish, middle tubule is thrown into a loop. The latter tubule communicates with the wide, large tubule by means of a distended part of the nephridial wall. This distended region is histologically differentiated from both the middle, brownish tubule, and the wide, large tubule. This has been named the *ampulla* by Gegenbauer.

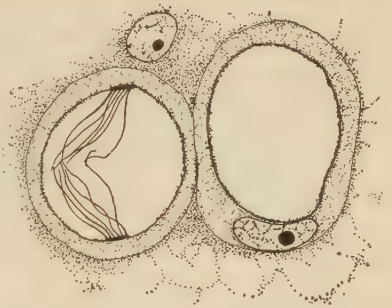

FIG. 307. — A transverse section through two regions of the upper, "narrow" part of the earthworm's nephridial tubule. One part ciliated. × 870.

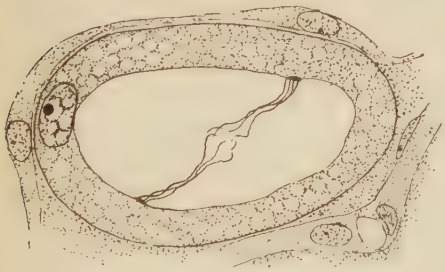

FIG. 308. — A transverse section of the brown region of the earthworm's nephridial tubule. × 870.

Two rows of cilia arranged in a slight spiral are found in the preseptal tubule; these cilia continue into the first part of the very long, narrow tubule, and occur at other points in this latter tubule. The middle, brownish tubule has also two rows of cilia throughout its course. The cytoplasm of the long, narrow tubule is finely granular. The nuclei are oval and smaller than in any other part of the nephridium (Fig. 307). The syncytium of the middle, brownish tubule is characterized by a heavy alveolar structure. The alveoli are most numerous near the lumen. The cytoplasm is more abundant and the oval nuclei of this tubule are larger than those of the narrow tubule. The rows of cilia lie opposite each other in the lumen (Fig. 308). The wall of the "ampulla" is sharply marked off from that of the brownish,

FIG. 309. — A transverse section of the "ampulla" of the earthworm's renal tubule. *ex.*, excretion particles. × 870.

middle tubule; on the other hand, it merges slowly into the wall of the wide, large tubule, so that no sharply defined boundary is formed. Its cytoplasm is differentiated into an inner and an outer layer. The inner layer is homogeneous except for excretion particles which it may contain. The outer layer of cytoplasm is distinctly striated. The striæ extend radially from the periphery to the inner layer of finely granular cytoplasm. The general striated appearance is distorted at places by excretion bodies and vacuoles (Fig. 309, *ex.*). The nuclei of this region are large and oval. They have the same general structure as those seen in Figures 308 and 310. This region of the tubule has apparently the power to elaborate excretion products. The syncytium of the wide, large tubule lacks the inner layer of "cell substance" or cytoplasm; otherwise it appears to be a structure similar to the "ampulla." Figure 310 was taken from a section near the "ampulla." Here excretion vacuoles and an excretion body are shown, *ex vac.* and *ex. gr.* These become less and less frequent as the tubule leads from the "ampulla." The striæ also become gradually less distinct farther from the ampulla. The muscular duct is well developed. Its diameter is about the same as that of the wide, large tubule. It is yet uncertain as to whether its lumen is intra- or inter-cellular. The cytoplasm of its wall is difficult to demonstrate. Certain sections may show it bearing as many as three nuclei in a single section; others show no nuclei and little cytoplasm. The muscles are distributed throughout the wall of the muscular duct in various directions. "The muscular duct penetrates the body wall, the muscles appearing to be continuous with those of the cuticular layer; a slight invagination of the epidermis meets the nephridial lumen, and puts the latter into communication with the exterior."

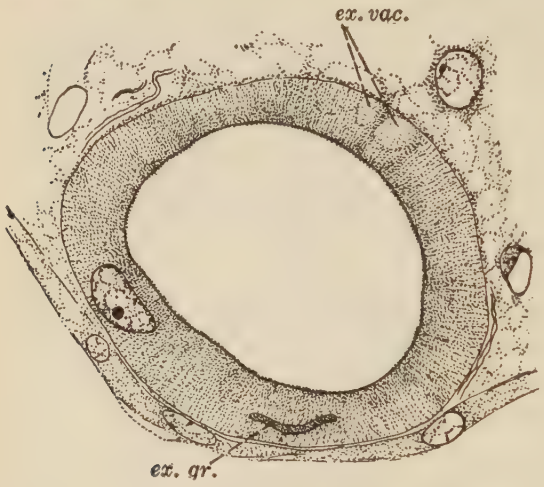

FIG. 310. — Sections of earthworm's tubule below "ampulla." *ex.gr.*, excretion particle; *ex.vac.*, excretion vacuole. × 870.

The closed nephridium of the Crustacea. — The nephridial tubule is free of solenocytes, but in the higher forms it becomes complex.

In the lobster, *Homarus vulgaris*, the nephridium has an end-sac and a middle convoluted tubule both of which eliminate waste products. The third and terminal region has been expanded to form a retaining vessel for the fluids excreted. The vessel delivers its contents through a pore to the exterior. The entire tubule is covered by a *tunica propria* of connective tissue, and is lined with a non-ciliated columnar epithelium. The cells of the end-sac are irregular and have the least dense reticular cytoplasm. Distally they bear large vacuoles in which excretion products are found. The nuclei are in the basal third of the cell (Fig. 311). The epithelium of the convoluted middle part of the

FIG. 311. — Section through parts of two regions of lobster's nephridium. *ex.p.*, excretory products; *ex.v.*, excretory vacuoles. × 425.

tubule is composed of stout cells with very distinctive features. The nuclei are spherical and have a conspicuous reticulum. There may be two nuclei in a single cell. The ends of the cells bear a well-defined cuticula. Beneath this there is a zone of reticular cytoplasm which tends to be striated toward the middle of the cell. The basal zone of cytoplasm is highly striated. These striæ lie at right angles to the base of the cell. Vacuoles containing excretion products are found within the cytoplasm (Fig. 311, at *ex. p.*). When these cells become highly active the cytoplasm at their free ends becomes highly vacuolated, and the cuticula becomes greatly broken in its contour. The cells lining the storage region or "bladder" of the tubule lack a cuticula. The vacuolization here is at the basal end of the cells instead of at the free ends. The marginal zone of cytoplasm is dense. Toward the base a

reticular structure becomes more evident until at the base there are many vacuoles. The cells are poorly defined (Fig. 312).

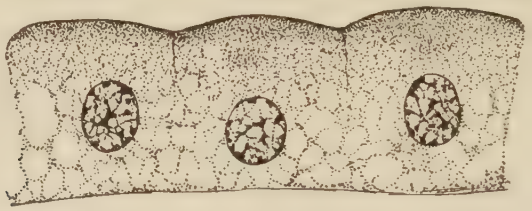

FIG. 312. — Bit of epithelium from the storage region or bladder of the lobster. × 800.

The nephridial tubule of the lizard, *Iguana.* — In this case the terminal wall of each tubule is invaginated by a vascular plexus to form a *Malpighian capsule.* The capillary plexus is known as a *glomus.*

FIG. 313. — Section of a glomus of the Iguana's nephridial tubule. *bl.ca.*, blood capillaries entirely filled with dense, nucleated, red blood cells; *ex.c.*, excretory cells; *c.n.*, capillary wall nucleus; *conn.t.*, connective tissue; *l.*, lumen of tubule (glomus). × 1500.

The glomus has an afferent larger arteriole which breaks up into a network of capillaries. This plexus of the arteriole forms a true *rete*

mirabile; i.e. it forms a capillary network connecting two vascular structures of the same character. The branching capillaries, before they leave the glomus, are reunited and the efferent vessel is formed, which is also an arteriole but slightly smaller than the afferent arteriole. The capillary plexus is supported by a slight framework of connective-tissue cells (Fig. 313). The glomus of *Iguana* measures about fifty microns in diameter. Figure 313 shows a section of a glomus from the kidney of this animal. The capillaries cut at various angles and filled with blood corpuscles appear in this section as dense bodies with a well-defined outline. Closely applied to this contour at places the flat nuclei of the cells of the capillary wall are seen in transverse section (Fig. 313, *c.n.*). These, except for their position, resemble much the nuclei of the terminal epithelium of the tubule. The nuclei of the connective-tissue cells are as a rule smaller than those of the cells of the capillary wall, and of the terminal epithelial cells (Fig. 313, *conn.t.*).

The terminal epithelium is composed of thin, flat, polyhedral cells which are continuous with the main portion of the tubule. After the tubule leaves the glomus its wall presents two types of cells histologically quite different. The tubule near the capsule is convoluted. This convoluted region is the larger of the two. Its diameter may be thirty-five to forty microns. The cells are stout columnar forms. In transverse section they appear as sections of truncated cones or pyramids. They measure twelve to fifteen microns in height. The inner margin of the cytoplasm has an irregular contour and tends to be striated. Beneath this there is a zone of finely granular cytoplasm which bears excretion particles. This zone occupies the greater part of the distal end of the cell. The basal or proximal part of the cytoplasm is more or less definitely striated and may contain vacuoles. The striæ are by no means as conspicuous as those shown in the nephridial cells of a frog (see Fig. 174). The nuclei are rounded and contain a distinct reticular network with small, rounded chromatin bodies, and one large chromatin mass which may inclose a nucleolus (Fig. 314).

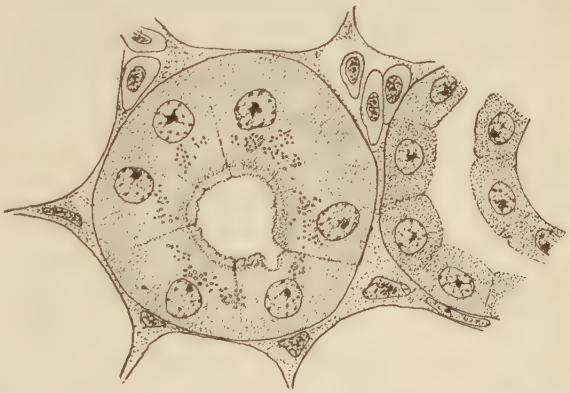

FIG. 314. — Sections of a thick and a thin region of the nephridial tubule of an Iguana. × 870.

The second part of the nephridial tubule is narrower than the convoluted portion; its diameter is about forty-five microns. The epithelium of the wall is composed of very low columnar cells which tend to be wider than tall. The cytoplasm is denser than that of the convoluted portion, and the cells have nuclei slightly smaller than those of the larger part of the tubule, but which have the same general structure. This smaller portion of the tubule most probably serves much less as an excretory structure than the convoluted portion. The smaller part of the tubule leads to and empties into a collecting tubule. The latter has an origin independent of the nephridial tubule.

In an embryonic stage it meets the nephridial tubule, and its wall coalesces with the wall of that structure. In Figure 315 we have shown at *A* a place of union between these tubules. The section unfortunately shows the cells cut obliquely so that parts of cells cut above their nuclei are seen lying to the right of the nucleated portions. Though the section

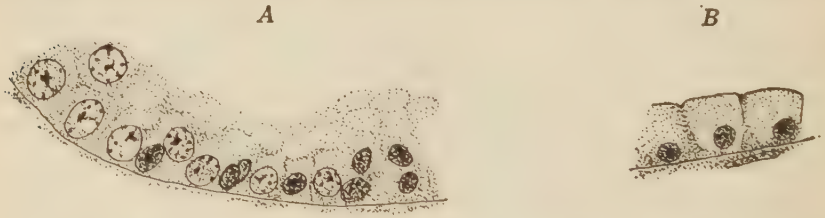

FIG. 315. *A*, section through the point of union of the epithelium of a collecting tubule and a nephridial tubule in the Iguana's kidney; *B*, some epithelium from the collecting tubule. × 870.

is not in this respect ideal, we may see the nuclei of the nephridial tubule, with their characteristic structure, interspersed with those of the collecting tubule. The cells of the collecting tubule become taller as they leave the nephridial tubule. At *B* in Figure 315 they are shown, seen in profile. The cytoplasm is finely granular and very clear at the distal end. At the base, and in some cases along the sides, a layer of denser cytoplasm occurs. The nuclei are dense and stain deeply. They are but eight or nine microns in diameter. The nuclei always lie near the base of the cells. These cells closely resemble mucous cells, while the cells of the nephridial tubule are suggestive of serous cells.

Examples of nephrostomes. — The nephrostome of *Polygordius* is comparatively simple. The intracellular lumen opens at the distal end of the nephridial tubule into the body cavity. At one margin of this opening is borne a tuft of cilia which is quite suggestive of the "flame" of a "flame-cell" (Fig. 316).

In *Perichæta malamaniesis* Benham, the nephrostome "consists of eight or nine marginal cells set in a circle around the terminal aperture

of the tubule. All the cells are equal in size, and each is ciliated over the whole of the centrally directed face, the other face being covered by a few cœlomic epithelial cells" (Fig. 317).

In *Lumbricus herculeus* the nephrostome has become most complex. The wall of the lumen of the preseptal tubule spreads upon one side to become fan-shaped; on the opposite side it thins out to become cleft. From this cleft on each side there diverges a row of grooved cells which are called the centrifugal cells (Fig. 318, *cf.*). These cells meet a second set of cells known as the centripetal cells (Fig. 318, *cp.*). The centripetal cells, as they are removed from the cells leaving the lumen of the tube, become large and form nearly a complete ring of cells about the end of the tubule. The cells of the ring have been called the marginal cells. These marginal cells are columnar. Their cytoplasm is slightly granular and supports a nucleus near its middle. Between the marginal cells and the centrifugal cells there lies a large, clear, crescent-shaped cell with a very large nucleus lying at its middle. This cell has been called the "central cell" (Fig. 318, *c.c*). Between its inner margin and the nephridial tubule the opening into the nephridial tubule is found. The marginal and grooved cells form an expanded collecting apparatus over which many cilia are distributed.

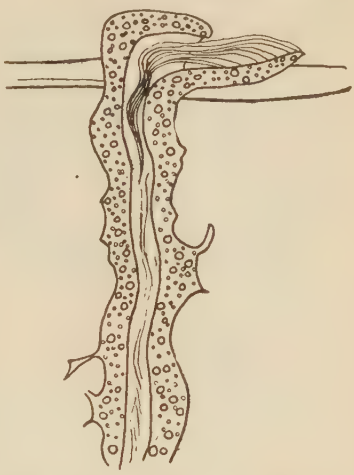

FIG. 316. — Section through the long axis of a nephrostome of *Polygordius*. (After GOODRICH.)

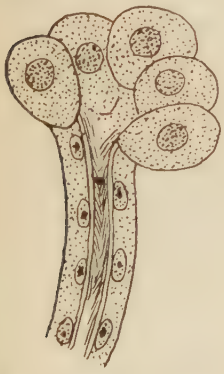

FIG. 317. — End of a nephrostome of *Perichæta malamaniesis*. (After BENHAM.)

In *Ammocötes* each tubule is provided with a ciliated nephrostome. The cilia are directed toward the nephridial tubule. The sides of the nephrostome are nearly parallel, so that the shape of the nephrostome is but slightly like a funnel. The cells forming its wall are small. They decrease in size as the lumen of the tubule is approached. Likewise the cilia decrease in size (Fig. 319).

Examples of structures of excretion accessory to tubules. — In the cœlomic epithelium of *Lumbricus* certain cells are found which have elaborated within their cytoplasm a substance which in picro-sublimate material has a coarse,

granular texture and a lemon color. They receive the name of *chloragogen cells*. This substance increases in bulk until the cytoplasm is for the most part filled by it, and the nucleus is crowded to the margin of the cell (Fig. 320, *A*, *B*, and *C*). Such cells frequently, if not always, when found in the epithelium, lie over a small capillary or space as indicated in Figure 320, *A*, *w.c.* These cells leave the epithelium to become wandering cells or amœbocytes. Figure 320, *C*, shows three such cells that had left the epithelium and were found lying in the cœlom clinging to each other as indicated in the figure.

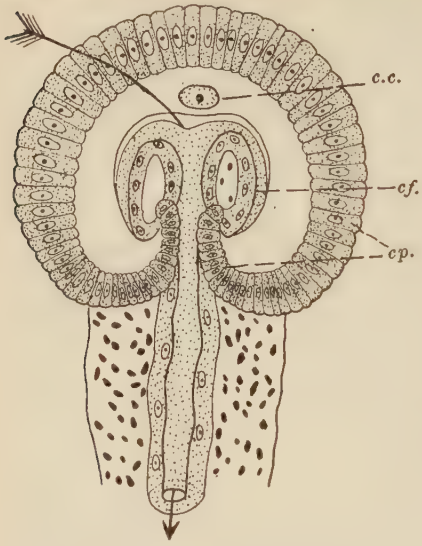

FIG. 318. — Nephrostome of the earthworm *Lumbricus herculeus*. *cf.*, centrifugal cell; *c.c.*, central cell; *cp.*, centripetal cells or marginal cells. (After BENHAM.)

In *Unio* **the epithelium of the pericardial cœlom**, in the region of each auricle, is thrown into folds to form a gland known as the pericardial gland. The tissues of this gland are in intimate contact with the walls of the heart, penetrating the tissues of the heart. By these *pericardial glands*, numerous wandering cells or amœbocytes are formed.

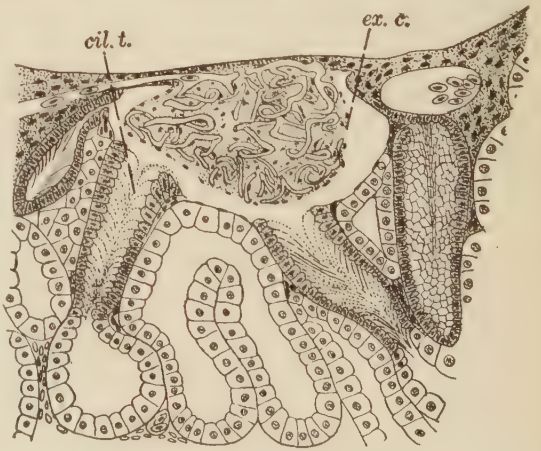

FIG. 319. — Transverse section through the kidney of *Ammocœtes*. One central glomus may be seen, covered with excretory cells (*ex.c.*); *cil.t.*, ciliated part of tubule. (After HALLER.)

These amœbocytes when they begin their activity are spherical to oval in shape. Their cytoplasm is rather dense and may contain one or more vacuoles. They are rather small at this stage, measuring about ten or twelve microns in diameter. The nucleus in the early stages of excretion activity is oval, with a diameter of about five or six microns. It

contains distinct chromatin granules of nearly uniform size (Fig. 321, *A*, *B*). The excretion product first appears as a small spherical mass of dense homogeneous substance, lying within the cytoplasm near the nucleus. This body continues to increase in size until it has become a large, dense sphere twelve microns or more in diameter. About this sphere the cytoplasm is applied as a thin film (Fig. 321, *D*). The nucleus is distorted and crowded to one side inclosed in a small amount of cytoplasm. The chromatin of the nucleus has become less distinct. The excretion or concretion sphere now breaks up into small bodies, which are scattered throughout the cell that has somewhat enlarged (Fig. 321, *E* and *F*). In the meantime, the nucleus shows a marked tendency to divide, and frequently amitosis is effected. The nucleus or nuclei finally disintegrate, and the cell has completed its course of excretory activity. The cells in this condition or their fragments leave the gland through the reno-pericardial duct to the kidney, and from thence to the exterior.

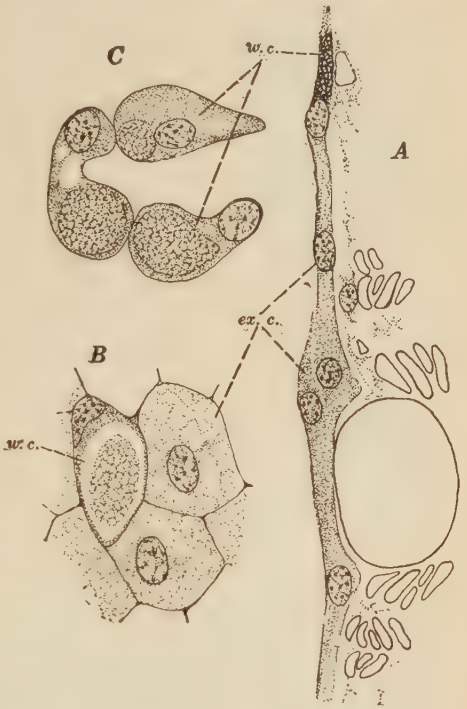

FIG. 320. — Epithelium from the cœlomic cavity of *Lumbricus*. *A*, vertical section through the epithelium with some of the underlying connective tissue, muscle, and blood vessels; *B*, surface view of three cells and parts of others; *C*, three wandering cells in the cœlomic cavity; *w.c.*, wandering cells; *ex.c.*, excretory cells. × 1300.

The chloragogenic cells of *Lumbricus* are found forming columnar

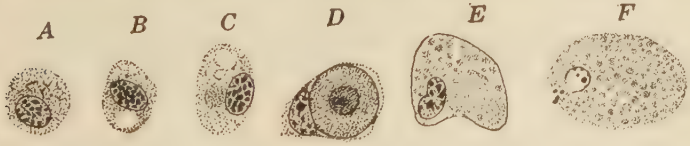

FIG. 321. — Six stages of secretion elaboration by the amœbocytes or "wandering cells" from the pericardial gland of *Unio*. × 870.

epithelium upon cœlomic surfaces. They are most abundant about the large blood vessels and the intestine. The typhlasole is filled with a

mass of these cells. They are tall cells with narrow bases and slightly expanded rounded ends. The cells taken from near the typhlasole measure one hundred microns or more in height. The cytoplasm is highly alveolar; in the distal part of the cell it is the denser. Many angular granules of chloragogen are usually held in the cytoplasm. The nuclei are small oval bodies lying near the middle of the cells. Each bears a single nucleolus (Fig. 322).

FIG. 322. — Several chloragogen cells from around the intestine of the earthworm. × 650.

The calcium phosphate cells of *Mesodon* are large, more or less conical cells, with their bases applied to the *membrana propria*, or basement membrane, of the epithelium. Their apices always communicate with the lumen of the hepato-pancreatic gland. From apex to base they measure thirty to thirty-five microns. The cells are isolated and surrounded on all sides by the hepato-pancreatic cells (Fig. 323).

The ureter and bladder serve chiefly as conducting and retaining structures. Because of this function their epithelium is compact and stratified. The basal layers of cells in these retaining epithelia tend to be columnar. These support a superficial layer of flattened cells which lie parallel to the surface of the bladder wall. The cytoplasm of the cells is dense. The nuclei are rounded and centrally placed (Fig. 324).

Beneath the epithelium in both the ureter and bladder is a connective tissue, *tunica propria*, supplied with scattered elastic fibers, lymphatics, and blood vessels. In both the ureter and bladder there is developed an outer coat, *tunica muscularis*. This also has a connective-tissue framework in which smooth muscle fibers are distributed in one, two, or three layers.

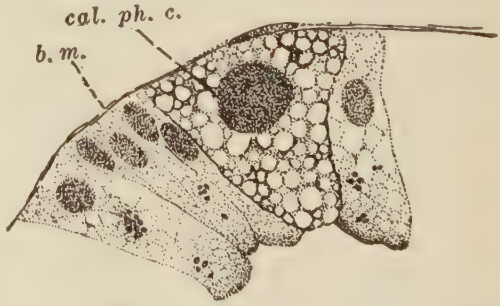

FIG. 323. — Cells from the digestive gland of *Mesodon* (*Helix*). *cal.ph.c.*, calcium phosphate cell. Others are hepato-pancreatic cells. *b.m.*, basement membrane, on which lies a narrow connective-tissue nucleus. × 970.

Technic. — We have here to do with a very easy tissue to cut and stain by the ordinary methods. Sometimes the vertebrate kidney, as in the mammals, and in *Petromyzon*, will prove refractory and get brittle, but a second attempt with a short fixation and careful treatment in the

water bath will usually give the desired results. If this fail, one can be sure of getting sections by the celloidin method, in which the difficulty of brittleness does not emerge.

In addition to the sections, it is very desirable to control the results of sectioning by a careful study of teased specimens. This method is vastly preferable to the use of reconstruction methods in the working out of long tubules, etc. One should complete no comparative study of the renal tissues without watching the live kidneys of a small annelid worm under the microscope. Other nephridia may also be studied in this way, in or out of the body.

FIG. 324. — Two basal cells in their natural relation to one superficial cell in the striated epithelium which lines the bladder of a mammal. (After KOELLIKER.)

To determine the nature of a suspected nephridial cell or tissue it is often possible to watch the cells excrete some foreign material, as carmine, which has been placed in the blood or body cavity of the organism. This may even be seen in sections taken the proper time after the material is injected.

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CHAPTER XX

THE INTEGUMENT, TISSUES OF MECHANICAL PROTECTION

The integument consists of the exposed outer portions of the covering epithelium of the body, added to, in nearly all cases, by certain connective tissue, muscle, and other cells that are located in its neighborhood. By "exposed outer" is meant such portions as are directly exposed to the medium in which the animal lives (air or water). The similarly constructed coverings of many internal cavities into which water or air are brought for respiratory or other purposes will be considered as a form of integument and not further differentiated from the former in this work except in so far as real differences exist between the two.

On account of the superficial position of an integument, its functions are most numerous. In the simplest and smallest organisms it performs most of the functions of the body. In the larger and more complicated creatures, many functions are still performed by portions of this surface layer, but these portions are removed from the surface by invagination to internal positions in the body, this happening in various degrees according to the conditions.

Although the surfaces used to perform the principal functions of the body, in the majority of organisms, have been removed from the outer integument, a number that cannot be performed internally have remained in it, and in addition to these are found minor duplications of some of those that have been removed to the inside. These latter have often acquired some secondary use in the integument.

With these functions as a chief basis for classification and with the aid of ontogenetic origins, we may, for convenience, classify the integumentary tissues as tissues of: —

A. Mechanical protection (and adornment).
B. Offensive mechanical protection and production of poisons.
C. Lubrication and cleansing.
D. The production of attractive and repulsive odors.
E. Adhesion and spinning.

Of the functions of the integument, mechanical protection is one of those that belongs peculiarly to it. We mean by the term a protection against *pressure, abrasion,* and the *entrance of needless or harmful fluids*

or other substances. Naturally, this work cannot be directly shared in by any other tissue in the body. The surface cells must perform it, assisted indirectly, in many cases, by the cells that lie next to and inside of them. The simplest way in which this work is done is by some kind of stiffening and hardening of the cells of the epithelium. We shall study this method both in columnar and stratified epithelia.

In the simplest columnar epithelia, this mechanical function is made evident by the lengthening of the cell, the formation of stiff fibrils in its cytoplasm (to offset pressure), the thickening of its outer border (to resist abrasion), together with the formation of terminal bars (to prevent the entrance of harmful or needless fluids).

These structures may be found performing their duties alone, as in the covering epithelium of some flat worms (*Planocera*), or in combination with many of the accessory structures to be described hereafter. They can be studied in a large number of the lower animals, and we shall first examine them as seen in the integument of a turbellarian worm, *Planocera folium* (Fig. 325).

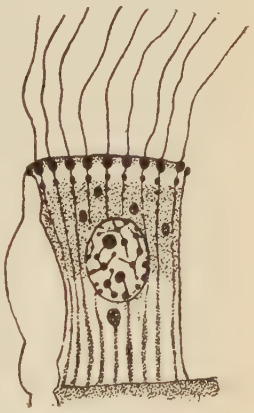

FIG. 325. — Protective and ciliated epithelial cell from the body surface of a flat worm, *Planocera*. (After SCHNEIDER.)

Here two of the features mentioned above are most excellently shown. The supporting function is performed by a series of stiff fibrils that extend from the proximal surface of the cell to its distal surface. Several granules may be seen on each of the fibrils near its outer end, and at the point where it leaves the surface a larger granule is placed. The fibril is apparently continued directly through this granule to form one of the cilia that, together with the other cilia belonging to the same cell and to the other cells, cover most of the body.

Thus the supporting fiber is evidently used for at least one other purpose than the more passive support of the cell. It is also used to support the moving cilium, and it is possible that it also has structural features that play a part in moving the cilium. It may even be conceived that other cell-organs have important structural relations with the fibril. Such ideas will not detract, however, from the conception of it as a cell-organ of mechanical support.

The fibril, so plainly seen as a straight support in this cell, is also found in many other epithelial cells throughout the different epithelia. These fibrils may be very slight, branched, net-forming, and otherwise variable, and in some cases hard to distinguish from fibrils of a totally different nature.

The function of resisting the entrance of foreign and unnecessary fluids is performed by two structures, the outer surface of the cell itself through the physiological processes that take place in or near that surface, and also by a series of *terminal bars* or "schlussleisten" found between the edges of the outer surfaces of the cells (see Fig. 47). These structures are rodlike and double, the two parts being closely applied and cemented to each other. Each half is structurally a part of the lateral cell-wall, and even when the lateral walls are separated one from the other, they remain connected by the closing-plates until the separating forces become very much stronger.

Any special structural device developed to perform the third protective function of resisting abrasion is not well shown in this example on account of the presence of the cilia, which render it unnecessary and impossible. Such a device usually appears as a thickened and hardened portion of the distal end of the cell, forming a platelike structure on the end of the cell. It may be seen to advantage in the digestive cells of the small intestine of vertebrates and in other places as on the outer epithelium of *Amphioxus* (Fig. 326).

FIG. 326. — Protective epithelial cells from the body surface of *Amphioxus*. The two kinds of cells represent two kinds of fixation as well, probably, as different physiological conditions. (First pair of cells after SCHNEIDER.) × 1000.

The next step in the development of the three functions under discussion, in a columnar form of epithelium, is the formation by the cells of an external and extracellular layer of some substance that will perform the functions better than the cells could themselves do. *Such an organ is the cuticle*, which is formed jointly by all the cells as a continuous layer covering the epithelium. In some cuticles, the portion formed by each single cell can be distinguished from that formed by the surrounding cells, but in most it cannot.

The cuticle is laid down by the cells in layers in most cases, or apparently as a single layer in some. It sometimes contains fibers, and in many cases it bears various points, knobs, and other structures on its surface. These structures may be the product of one cell or of many cells. Various openings, usually of small size, serve as a means of exit for the secretions of glands and the cilia and nerve-endings of other cells as well as to permit odors to reach some olfactory cells.

The cuticle is often strengthened by the addition of mineral salts and the addition of foreign substances to the exterior. It is also renewed in most forms either by small parts at a time (leech), or by a process of entire shedding of the whole structure at once (lobster). In this case

its place is taken by a new cuticle that is usually formed before the old one is removed.

The substance of the cuticle is an organic material called *keratin*, which varies somewhat in the different forms. It is, in some cases, said to be made of cellulose. The cuticle found on many worms is a simple type, of moderate development, and that of the earthworm will do for examination.

The cuticle of the earthworm is a layer of material about one eighth or one tenth of the thickness of the layer of epithelial cells. Its structure does not appear to advantage in a section, and it is best studied in a piece of the layer that has been torn or macerated off in alcohol of 30 per cent (Fig. 327). Here it is seen that the whole structure of this organ consists of two parallel series of fibrils, each set lying at right angles to the other, and all flattened together by a cement substance that fills all the interstices. At somewhat regular intervals the fibrils are pressed somewhat apart by a greater amount of the cement substance, and through this thickened cement may be seen a fine pore passing through the entire layer. This pore is seen in transverse sections of the cuticle *in situ* on the epithelium, to provide a passage for the secretion of one of the many unicellular mucous gland-cells that are to be found at numerous intervals on the surface of the body.

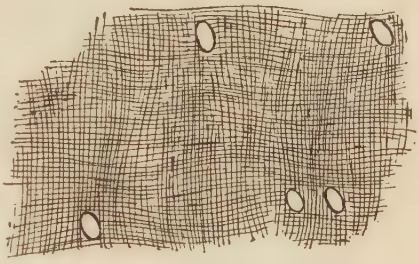

FIG. 327. — Superficial view of cuticle from an earthworm. × 1500.

Certain regions of the cuticle show spots that provide many similar pores set closely together. Again, the section of the entire integument will show that these are the passages through which the perceptory endings of a number of nerve cells come to the surface where they will be in contact with the outer conditions.

The fibrils that make up the bulk of the cuticle are straight and parallel, and run, as has been said, in two series, the strands of each series being at right angles to the strands of the other. Both series are symmetrically oblique to the main axis of the animal's body, and are thus at an angle of forty-five degrees to it. The fibrils are thought to pass from one surface to the other, and to interweave. It is puzzling to understand how the epithelial cells that are arranged all over the surface of the body, and in no particular order, are able to coöperate in such a manner as to lay down or form such long, straight, and parallel fibers in two distinct sets.

The exact method by which the cuticle is laid down by the cells has

not been especially worked out in the earthworm. Also the renewal processes which must take place to repair the loss by attrition in this hard-burrowing animal are not understood. For methods of cuticle renewal, see the following description of this process in the Crustacea. The two have, doubtless, much that is similar.

The lobster is also an animal that has an epithelial layer of cells on the outside of its body and a cuticle covering these cells. The columnar cells composing this epithelium are large and well formed, and vary much as to length and the development of their characteristic organs, which are the same, however, and easily distinguished wherever seen. The supporting fibrils are particularly well seen, and are very instructive because of the fact that they are to be divided into two groups according to whether muscles are attached to the cells to which they belong or not. In Figure 328 a portion of the epithelium of a lobster is represented with the cuticle lying on its upper surface and two kinds of supporting fibrils represented in the cells. The cells on the left are from a region where the fibers of a muscle are attached, and the fibrils of this muscle can be seen so closely connected with the fibrils of the epithelial cell that they seem to be direct continuations of them. The supporting fibrils are, in this case, made strong and straight so that they can bear the strain of the contraction of the muscle. In the adjoining half of the epithelium, in the right of the illustration, it can be distinctly seen that the supporting fibrils of the cells are not called on to withstand any such strain because of the blood space

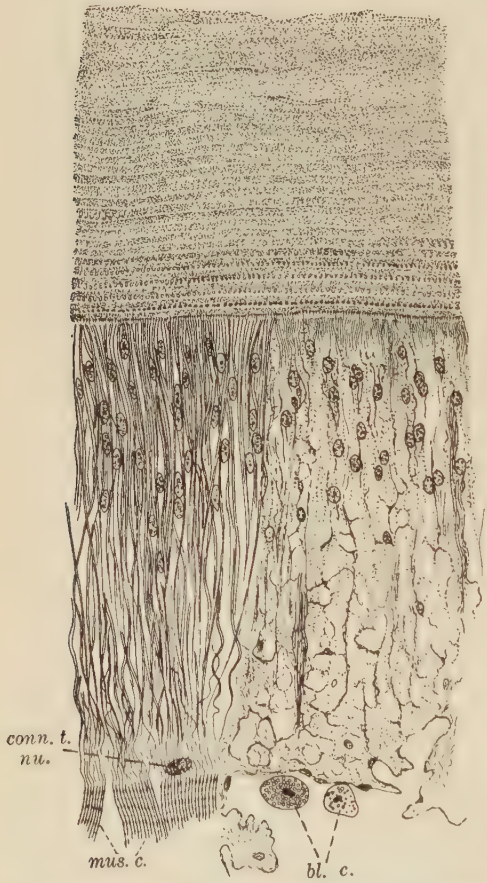

FIG. 328. Portion of the new integument of a lobster, *Homarus*. *conn.t.nu.*, connective-tissue nucleus; *bl.c.*, blood cells; *mus.c.*, muscle cells.

that bounds their proximal surfaces, and here, accordingly, we see no such strength and straightness of the fibrils. They are somewhat branched in these cells and of much more delicate formation.

This epithelium is also interesting because of the fact that connective-tissue cells have wandered among the bases of the cells, and because it is an example of an epithelium without a basement membrane. The lateral boundaries of the cells are very thin and very difficult to see. As in the case of other epithelia that are used to form a shell of lime, the nuclei of the cells are nearer the distal end of the cell than those of the majority of other epithelial cells. This latter fact is particularly true of the plecypod mollusks.

The cuticle that we find in the earthworm is represented in the lobster by a thick structure, the shell, which is a real cuticle of organic material, stiffened and hardened by the deposition of salts of lime. The organic base of the shell consists of a square-meshed reticulum built up of fibrils (see Fig. 329). In the meshes of this reticulum is a more weakly developed groundwork of organic substance, the whole plastic structure being combined with the lime salts much as it is in bone.

The specimen that we are studying is a section of the integument of a lobster that had just shed its shell, and had not yet had the lime salts deposited in the new one. Thus it may be assumed that we have the best view obtainable of the organic structure of the shell, and it can be seen that it is stratified, with the strata somewhat thicker and less distinct the farther they lie from the cells. The darker parts of the substance that are thinnest, and which serve to separate the strata from each other, are the horizontal lines and meshes of the reticulum, and are much easier to distinguish than the vertical lines. The layers thus seen are to be grouped into a number of regions in the thickness of the shell. The best grouping of these layers seems to be into an outer and very thin region that is denser and darker than the rest of the shell, a somewhat thicker

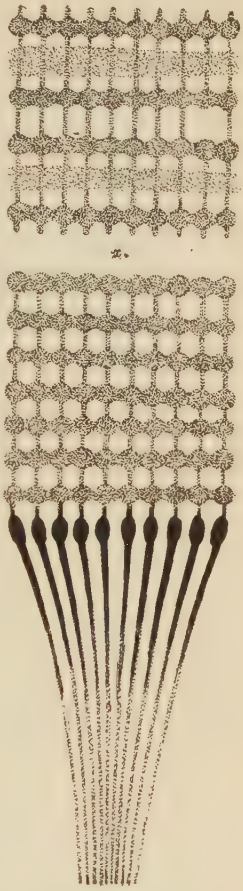

FIG. 329. — Slightly schematic figure of the structural groundwork of a lobster's shell. The figure is discontinuous at *x*, and incomplete on the upper surface for want of room. (After SCHNEIDER.)

middle region that contains the pigment, and an inner or principal region that seems to serve as the chief means of mechanical support. The strata of the inner region are hardest in the outer portion, and become softer as they are examined nearer the cells. The innermost is but weakly calcified.

In general, the cuticle, in its office of mechanical protection, is often found to bear modified portions, as lumps, ridges, knobs, hairs, spines, etc., of complicated patterns and varying sizes. The simplest form of this modification is where a cuticle-like structure that ordinarily extends in an unbroken sheet over the surface is more highly developed in certain locations, forming isolated areas, rods, cones, spikes, or one or another of an almost infinite variety of structures scattered more or less regularly over the surface of the integument. In all these cases the structures in question are formed like other cuticle by the action of the epidermal cells. Sometimes one, sometimes many, of these cells form each such a structure, and frequently the structure acquires a secondary use. The strange cuticle-like structure found in the gizzard of the bird, the stomach-jaw of the Arthropoda, and the other organs of mastication of a cuticular nature have been treated of under the tissues of mastication (Chapter XV).

Turning our attention again to protection against abrasion by an epithelium, we find that *stratification* (Chapter VI) is, in its essential features, **a highly developed means of mechanical protection.** The structural devices that a stratified epithelium develops to accomplish its ends are much the same as in the simple epithelium. They are simply modified to meet the different structure of this form of epidermis.

A cuticle is not formed as an extracellular structure in the stratified forms of epithelia. But the outer cells of the epithelium are modified so greatly that they form layers of different structure and consistency that are admirably adapted to all purposes that a cuticle could fulfill. Only in this case it is the cells themselves that perform the work, and not an extracellular material that they have formed. The similarity of the two processes is much heightened by the fact that the cells that do this duty are dead themselves and might be compared very closely with a cuticle from the standpoint of function, far removed as they are from it in their origin. This form of outer protection is also somewhat more convenient than the cuticle of a simple epithelium because it does not have to be shed at intervals, except in a very few cases, but is continually and gradually dropped and renewed. For **a very simple sort of stratification** see Chapter VI, where this is illustrated in a chætognath worm *Sagitta*. An example of this principle, carried to a large degree of efficiency without any great specialization, is furnished **in the skin of man.** This is pictured in Figure 330. A small region of this epidermis is here

shown resting on a portion of the underlying mesodermal tissues called the *derma*. This derma is a part of the skin, being especially set apart from the more central tissues and strengthened to afford a strong and elastic bed for the epithelium. A *basal membrane* is present, and upon its slightly curved surface rests the basal layer of the epithelium. This layer is made up of a very perfect layer of slightly columnar cells whose nuclei are oval, probably on account of their somewhat crowded condition. They lie in the cell at some little distance from the basal membrane, and are frequently met with in mitotic division.

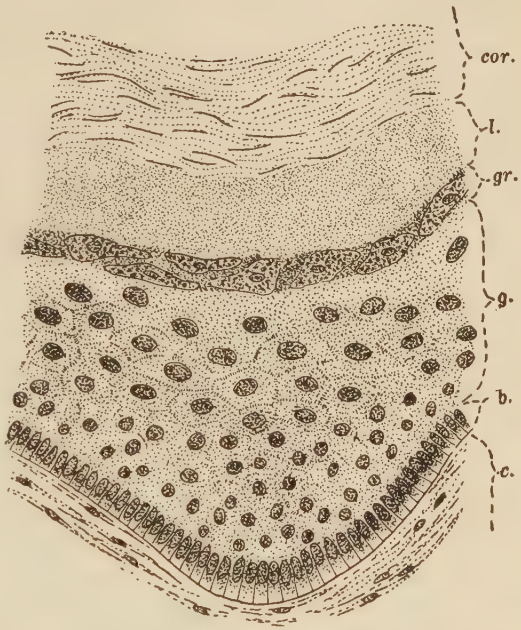

FIG. 330. — Portion of a vertical section through the epidermis of man. *c.*, part of the underlying corium; *b.*, basal layer of the epithelium, separated from corium by the thin basement membrane; *g.*, stratum germinativum; *gr.*, stratum granulosum; *l.*, stratum lucidum; *cor.*, stratum corneum. × 350.

The many cells derived from this basal layer lie above it in a far thicker layer of cells that takes up about one half of the entire thickness of the epidermis. This is known as the *stratum germinativum*. Most of the cells in this layer show evidences of an amitotic division, a terminal process in the life of the cell. The nucleus and cell both grow in size until, at the outer boundary of the layer, they are transformed by a deposit of granular matter into the cells of a thin granular layer, known as the *stratum granulosum*. This layer is about two cells thick, and its flattened nuclei show signs of degeneration. The layer stains rather deeply.

Above this stratum the cells are changed, first into a homogeneous and non-staining *stratum lucidum*, and then by a stratification and hardening as well as flattening, into an outer *stratum corneum*. Both of these two last layers are dead cells which are toughened and developed to act as buffers between outer abrasion and the delicate and living tissues beneath them. As fast as any of these cells are rubbed off, new ones are added from beneath. A cell from this outer layer is large and thin an l

has a flat, round, and non-staining nucleus. Its strength comes from a deposit of a substance called first *kerato-hyalin,* which then changes into *eleidin,* and that into *pareleidin.*

Just as the cuticle of the columnar epithelium is developed into a variety of organs, so also is the outer protective layer of toughened dead cells in the stratified epithelium developed into a vast number of structures that are used for a variety of purposes. In a very general way it may be said that these structures represent and are made out of the same kind of outer cells that cover the surface of the stratified epithelium. They become so hardened and fitted together, however, that it is difficult to distinguish them as cells. These structures are usually developed from a portion of the epithelium that has been invaginated.

One of the commonest and simplest forms of this structure is an evagination, and is to be found on the tongue of most mammals, especially on those which eat living prey. The upper surface of the tongue is evaginated into a series of close-set papillæ covered with their stratified epithelium. In the hollows this epithelium is thin and soft. On the pointed tips it is immensely thick and strong, and is so hardened and compacted that the animal can use its tongue as a rasp to scrape meat off a bone.

The same sort of development on flat areas separated by valleys lined with a softer epidermis results in the "scale" of the reptiles. The legs of birds show such structures well developed, and in the entire skin of a fowl or pigeon early rudiments of this formation may be seen. Such scales are shed periodically and replaced by new ones which are developed beneath them. The method of fission between the old and new layers is unexplained. It can be well seen in the snake.

It is the analogous structures which are developed from invaginated regions of an outer stratified epithelium that show the most perfect organization, however. Such are the hair and feathers of the mammals and birds. Of these two, the feather seems to be the highest specialization.

Hair is formed in general as follows. — It begins in the embryo mammal as a thickening of the epidermis, particularly of the basal layer. This thickening soon develops into an invaginated pocket filled, of course, with the basal cells. This pocket deepens into a long tube whose fundus is widened and filled with the cells of a considerably thicker epithelium than that which lines the sides. The bottom of this bulb is then evaginated for a short distance by the growth of a mesodermal papilla, and the epithelium on this papilla begins to grow much more rapidly than any of the rest, forcing a pointed mass up through the lumen of the tube.

This mass consists of a central core which is the *hair shaft* with an outer layer called the *inner sheath.* This sheath travels with the hair

shaft and slides against the cells of the epithelium which lines the hair tube. These latter form the several layers of the *outer hair sheath* which is thus a modified form of stratified epithelium continuous with that of the surface. At about two thirds of the distance to the surface the inner sheath degenerates, and the hair is surrounded directly by the epithelial layers of the outer sheath.

This epithelium, at two places, about a third to a half of the distance from the surface to the bulb, is thickened by the growth of its basal layer. The upper of these is the developing *sebaceous gland* which is described

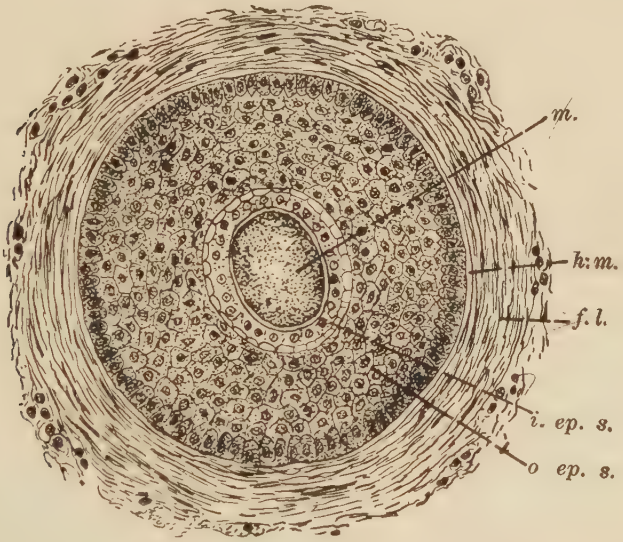

FIG. 331. — Section of a hair root, just under the skin. *m.*, medulla; *i.ep.s.*, inner epithelial sheath, *Henle's layer* and *Huxley's layer; o.ep.s.*, outer epithelial sheath; *h.m.*, hyaline membrane; *f.l.*, fibrous layer.

under lubrication. The lower is a center for the renewal of the hair, which falls out after its term of usefulness is over (Fig. 331).

The layers of the outer hair sheath are interesting. The upper is a continuation of the outer epidermis. The *stratum corneum* extends down as far as the sebaceous gland, where its place is taken by the inner sheath. The *stratum granulosum* extends farther, and only the basal layer can be traced continuously to the papillæ.

In the birds an epithelial structure is produced, somewhat similar to hair, and **called the feather**. Like the hair, the feather begins in the embryo as a thickening of the very thin epithelium which covers the integument (Fig. 332). The same layers are found here as were seen where the hair developed in the mammal, with the small differences of detail, that in the hair anlage a thicker layer of epithelium is to be

found, and not so pronounced a thickening of the mesodermal rudiment of the papillæ as is to be seen in the feather anlage.

The feather is very complicated in its development, and we shall describe the completed structure first (Fig. 333). It consists of a more or less long quill which is set in a follicle much as a hair is. The quill is a hollow tube in which is usually found a loose parchment-like series of irregular lamellæ which divide it into several chambers. At its proximal extremity, in the bottom of the follicle, it opens through a slightly constricted neck, and a mass of vascular mesodermal pulp, covered with the stratified epithelium of the follicle, reaches up for a distance into its lumen. This structure is the *feather papilla*, and the opening is known as the *interior umbilicus*. The quill rests against the sides of the follicle which are covered with the invaginated stratified epithelium. Distally, the short quill is extended into a longer and somewhat smaller shaft known as the *rachis*. At the point of juncture there is another small opening, the *superior umbilicus*.

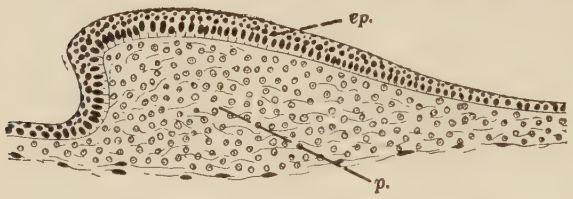

FIG. 332. — Very early rudiment of a down feather in a pigeon. *p.*, papilla; *ep.*, epidermis. (After DAVIES.)

The rachis has a solid wall with an alveolar core, and from each of its two sides springs a longitudinal row of more closely set, parallel, thin plates called the *barbs*. Projecting again from the upper or distal edge of each barb are two rows of very small processes known as the *barbules*. The anterior row of barbules is provided on its lower side with a series of tiny hooks, while the posterior row is shaped into a series of tiny, ragged-edged plates so placed that the anterior barbules of the next barb will catch in them and hold as strongly as their elasticity will permit. When forced apart, the hooks, if uninjured, will catch again the next time they touch the plates of the neighboring barbule.

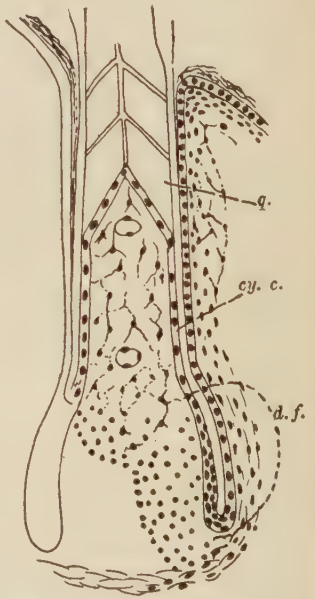

FIG. 333. — Lower portion of a nearly completed down feather in the pigeon. *q.*, quill; *cy.c.*, cylinder cell layer; *d.f.*, beginning of the growth of the definitive feather. (After DAVIES.)

All these structures, the quill, rachis, barbs, and barbules are dead

cellular matter derived from the epidermis of the feather papilla. When the feather is pulled out or is molted, the papilla is left behind, and

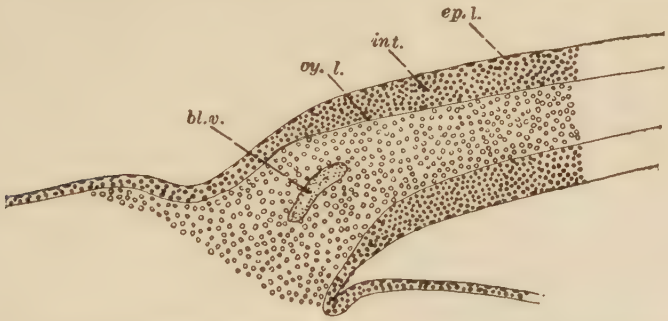

FIG. 334. — A second stage in the growth of a down feather. *ep.l.*, epitrichial layer of epidermis; *int.*, intermediate layer of epidermis; *cy.l.*, basal layer of cylinder cells of epidermis; *bl.v.*, blood vessel in papilla. (After DAVIES.)

begins to form a new feather by the growth from its proximal dorsal surface of a new papilla.

The first feather to be formed by the embryonic papilla such as was mentioned on the preceding page is called the *down feather*. The embryonic papilla (Fig. 334) elongates, the while that it settles into the skin. The whole structure now consists of an evaginated region of the bottom of an invaginated region. The outermost epidermal layer, however, does not dip into the fundus but reaches from the surface directly up and over the feather rudiment.

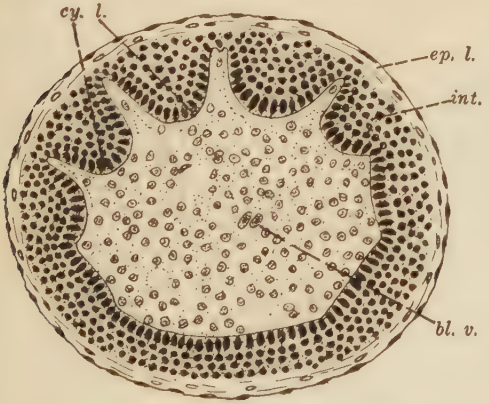

FIG. 335. — Transverse section through a somewhat older pin feather than that represented in Fig. 334. *bl.v.*, blood vessels in the papilla; *ep.l.*, epitrichial layer of epidermis; *int.*, intermediate layer of epidermis; *cy.l.*, basal layer of cylinder cells. The longitudinal cylinders are partly of cylinder cells. The longitudinal cylinders are partly formed on the dorsal side of this young structure. (After DAVIES.)

The epidermis is very thick on the papilla, and soon begins to show a differentiation. The basal layer is thrown into longitudinal folds whose inner flexures are round, and whose outer folds are sharp and plate-like (see Fig. 335). The mesodermal tissue of the papilla extends as a thin layer out into the plates. The middle and basal layers of the epidermis are now divided into a series of *cylinders* running lengthwise on the papilla.

The two or three outer layers of the epidermis not involved in this cylinder formation now form a stratified and partly cornified layer, the sheath layer, on the outside of the rudimentary feather (Fig. 336). The outer edges of the plates, where they touch this sheath, become flattened and broadened to partly cut the cylinders off from the sheath layer. This is more apparent distally and disappears proximally.

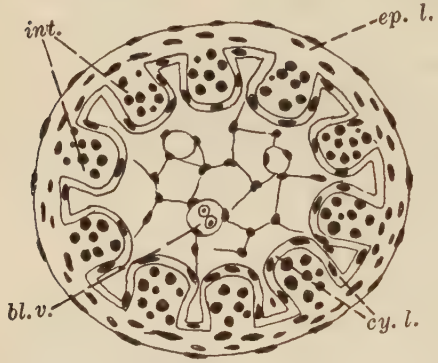

FIG. 336. — A later stage, than Fig. 335, in the development of a down feather of the pigeon. The cylinders are well defined, but have not yet cut off their inclosed intermediate cells, which now form the *longitudinal plates*, from the epitrichial layer. Letters same as in last figure. (After DAVIES.)

Finally the cylinder cells form concentric layers in each cylinder, and cornify in the distal part (Fig. 337). The sheath falls off and the pulp tissue withdraws its blood supply and dries up. This leaves the cylinders free, but still connected at their basal ends with the hardened base of the papilla which has not formed this part of its epidermis into cylinders. We thus have, as a completed structure, a quill-like base from whose distal end arises a circular row of filamentous processes called the *barbules*.

This *down feather* is soon lost, usually by being pushed out by the new or permanent feather which takes its place. This second feather, which like all its successors is known as a *definitive feather*, arises as a new papilla that grows out of the base of the old one which it reabsorbs. Like the first papilla, it is a dermal pulp, covered with a thick epidermis. This epidermis, when the papilla is large enough, begins also to develop a series of longitudinal folds much like those of the down feather rudiment. Several of the folds on the dorsal side of the papilla continue straight and become

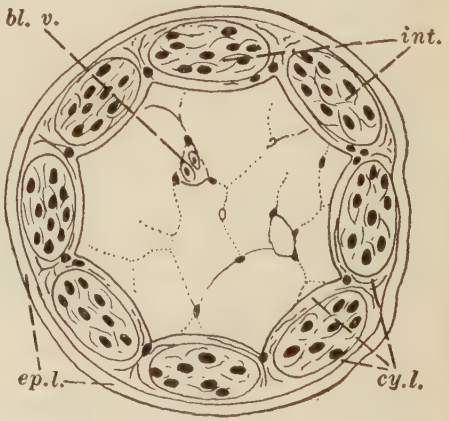

FIG. 337. — Transverse section of a young down feather near maturity. *int.*, longitudinal plates of what were formerly the intermediate cells; *cy.l.*, cylinder cells which now surround the longitudinal plates. Other lettering same as in preceding figure. (After DAVIES.)

fused together as one. They grow extensively and become the rachis. Figure 338 shows a cross section of a feather that shows this development.

The outer cylinders become arranged obliquely on the papilla pulp so that they form two series each attached in a line, one to one side, the other to the other side of the rachis. They now lie obliquely on the papilla, and as the rachis emerges from the skin it drags them in two rows with it. They continue soft and developing on the bottom until the head of the quill emerges with the last of them, thin and poorly developed, as a ring of downy barbs which surround the superior umbilicus.

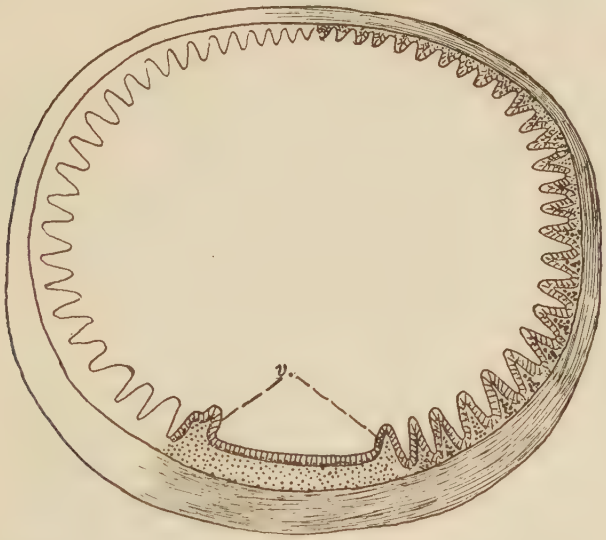

FIG. 338. — Transverse section of a large vane feather or permanent feather in an early stage of development. *v.*, point at which two of the longitudinal plates are united and enlarged to form the *vane* or *rachis*. The remaining plates become specialized to develop the barbs and barbules of the feather. (After DAVIES.)

The growth of the individual cylinder into a barb of the definitive feather is complicated by the fact that the latter develops, in the course of its growth, the two series of barbules on its sides. It is the inner rod-like region of the cylinder which hardens into the barb, while its outer cells become arranged in slanting rows and harden into the barbules, forming the hooks in one set and the plates in the other. Space forbids us to go further into the details of this complicated development which has been worked out by H. R. Davies and others. Figure 339 shows a transverse section of a single barb in process of development.

Scales of Fishes. — Our last example of an integumental structure used for mechanical protection is peculiar, in that it is developed in the

mesodermal element of the skin below the epithelium which takes no part in its formation. This structure is the *scale* found on the body surface of the teleost fish.

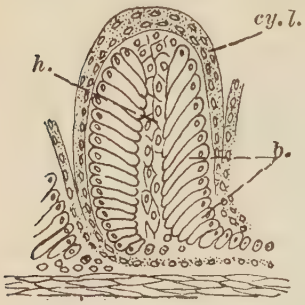

FIG. 339. — Parts of two longitudinal plates from a permanent feather somewhat more advanced in development than in the preceding figure. One of these plates of cells will produce a single barb with its double row of barbules. *cy.l.*, cylinder layer of cells; *b.*, cells which will form the barbules; *h.*, horny covering. (After DAVIES.)

The skin of a fish consists of a thick layer of connective tissue laid down in thin horizontal layers and forming the base on which rests a heavy, stratified epithelium. In the embryo these layers are distinctly separated from one another by a clear, sharp-cut line, the basement membrane. The outermost layer of the connective tissue, or cutis, changes from the flat, highly specialized connective-tissue cells to rounded mesodermal cells with oval nuclei whose chromatin is arranged in a pattern that resembles the arrangement in the nuclei or epithelial cells. This layer slowly increases by mitotic divisions. Later in the development (trout of forty-five millimeters), it can be seen that this layer has become divided into two layers by a deposit which the cells are laying down between them (Fig. 340). This layer of homogeneous and dense material represents a longitudinal section of a thin oval plate called the *scale*. It increases in size (which is represented by length in our drawing) and also in thickness.

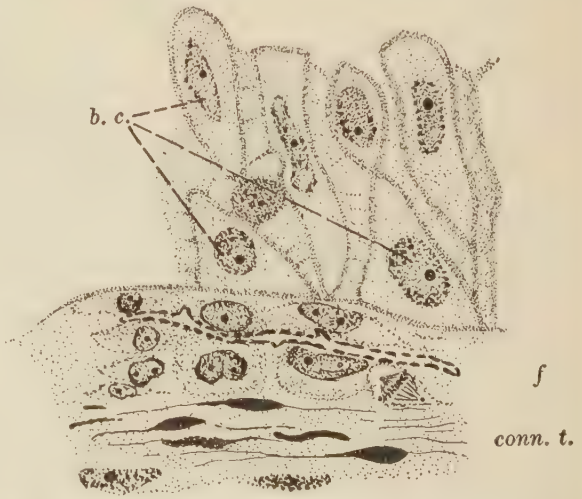

FIG. 340. — Several basal epithelial cells (*b.c.*) resting on the rudiment of a young scale of an embryo trout. *conn.t.*, unmodified connective tissue of the corium; *f.*, the two distal layers of connective tissue constituting the scale follicle. The young scale, in section, is indicated by a dotted line. (After NUSBAUM.)

These scales occur, placed in a regular pattern, over most of the fish's integument. At first they do not interfere with one another, but later they increase so in size, especially on the posterior edge, that they overlap, and come to lie

as a series of alternate and overlapping plates, like the shingles on a house. They push the posterior part of the connective-tissue pocket in which they lie, up into the thick epithelium from which they are separated by but a thin layer of connective tissue and their own matrix cells.

These matrix cells of the papilla, which were at first round and plump, now become drawn out into a very thin epithelial-like layer. Those on the upper side of the scale lay down as a rule a more uneven surface on the scale than those in the lower layer. In many fishes the upper surface, especially on its posterior part, is covered with many points, knobs, or other processes which cause it to be called a *ctenoid scale.* During its

FIG. 341. — Two scales near maturity in the skin of a young trout. *b.m.*, basement membrane of the overlying striated epithelium; *conn.t.*, connective-tissue corium; *f.*, scale follicle formed from connective tissue of corium. The mesodermal formative cells lie on the distal and proximal surfaces of the scales, forming a very thin layer on each surface. (After NUSBAUM.)

earliest stages the young scale lies almost flat or parallel with the body surface. As it grows in extent, the posterior end is tilted up. In Figure 341 the shrinkage of the layers has caused the scales to stand up much more than they do in life. The ganiod and selachian fishes also have mesodermal structures developed in the skin for protection.

The integument of the echinoderms also shows a set of hard plates consisting of a deposit of lime in its tissues. This series is another example of the comparatively rare cases in which these mechanical protective structures are developed in the *cutis.*

The epidermis in these animals is a thin, simple epithelium, and not fitted to stand either abrasion or pressure. The underlying plates of the cutis not only protect the body from pressure by their rigidity but they also protect the epidermis from abrasion by the formation of outlying

points which loose their own epithelial covering, but succeed in preserving that of the wider surfaces which lie between them.

The shape of these plates varies exceedingly. Some are flat and hard. Others are spongy in texture, while in the Holothuria they form isolated plates of various patterns, some of them very beautiful, as the anchor plates in some of the *Synaptas*.

Technic. — The technic of this group of tissues varies from the simplest and easiest to the most difficult, mechanically, owing to the great variety in which the protective substances and cells are developed. The difficulty lies in the hardness of these parts, and the consequent breaking, irregularity, and unmanageability of the sections. The heterogeneous tissues are the worst in this respect. The remedy in most cases is an extra sharp and good knife, and a deliberate and careful handling of the sections when they have been secured. In case the hardness is due to some salt of lime, the tissue should be decalcified either by the fixative or by a subsequent treatment with hydrochloric acid and phloroglucin. When it is due to chitin and connective-tissue substances the problem is not so easy. Most methods of softening these materials injure the structure and staining power. It is therefore better to use such processes only as a last resort and to first try to get sections with a very good knife by the ordinary way. Sometimes it is best to saw off thick sections and then to grind them down on a stone in oil. If soft parts are associated with the hard parts and must be preserved, the whole mass must be fixed and then embedded in rosin, in which condition the entire structure, soft and hard alike, may be sawed off and ground and then mounted after the rosin has been dissolved. In some instances teasing of fresh or fixed material and hand sections of fresh material will give good results after all other methods have failed.

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OFFENSIVE MECHANICAL PROTECTION AND POISONOUS FLUIDS

Offensive mechanical protection is a function of many kinds of epithelia and the products of these epithelia as well as dermal structures associated with them. It is but remotely removed, so far as the structure of the tissues that perform it is concerned, from the passive, mechanical protection treated of in the last section. A series of merely ornamental points on an insect larva might be developed by selection or otherwise into stinging organs and spines. Also the same sort of development of the hardened outer layer of the stratified epithelium that results in a downy hair or feather is sometimes used to produce claws and horns. The principal factor that leads the writers to separate these mechanically offensive organs from the simple protective structures, histologically, is the fact that many of them are associated with poison glands. The associated poison glands will also be described at the same time.

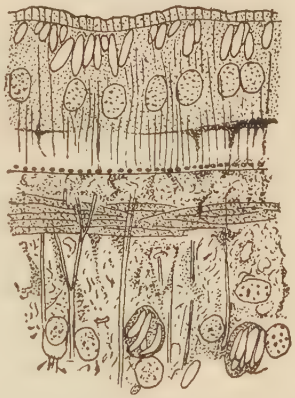

FIG. 342.—Part of a vertical section of a triclad worm. The outer layer consists of a simple layer of columnar, epithelial cells containing trichocysts in their distal cytoplasm and showing a modified and protective border. Beneath this the underlying tissues have become arranged in layers with reference to the body surface. (After PARKER and HASWELL.)

We shall first consider three intracellular forms; the *trichocysts of Infusoria* (see Fig. 245); the *rhabdites of turbellarian worms*, and the *nettle-cells of cœlenterates*. Secondly, the *extracellular forms from columnar epithelia* and the *multicellular forms found developed from stratified epithelia.*

The simplest forms of such organs are the **rod-like trichocysts of** *Paramœcium* and other Infusoria, and the so-called **rhabdites and stylets found in the turbellarian and nemertean worms** (Fig. 342). These are short rods pointed at the outer end and formed in the cytoplasm, usually of the cells that contain and use them. They may be formed by internal

cells in the higher forms, however, and passed from cell to cell until delivered for use at the surface. It is probably an organ of defense, and is usually surrounded by a layer of slime that may contain poison. The rhabdites are developed in some cases into large structures called stylets and, by some, these are thought to be a transition from the rhabdite into the next form of offensive mechanical protection that we shall study, **the nematocyst or nettle cell of the cœlenterates.**

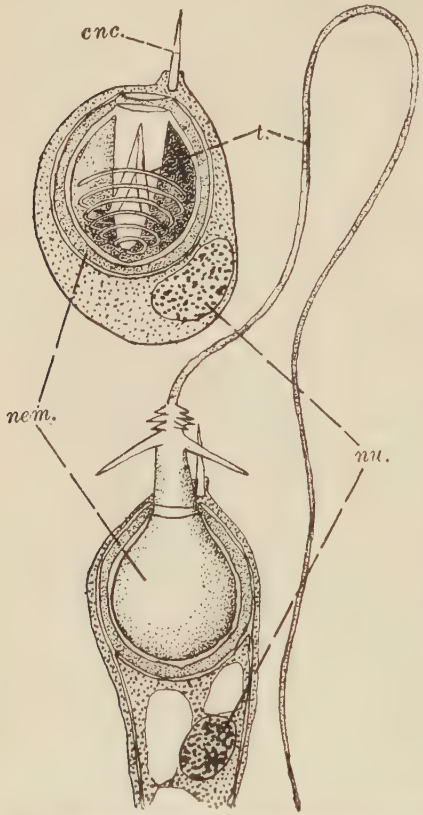

FIG. 343. *A*, young but fully formed cnidoblast of *Hydra*; *B*, freshly discharged cnidoblast of same animal; *t.*, thread; *nem.*, nematocyst; *cnc.*, cnidocil; *nu.*, nucleus of cnidoblast. (After SCHNEIDER.)

The nettle cell or *cnidoblast* is an organ that is developed in the cytoplasm of a single cell, and is a marked example of a regular and complicated structure of great efficiency and delicate adjustment formed by an apparently amorphous mass of protoplasm. It much resembles the *trichocyst* of some Infusoria with which it is probably homologous (see Fig. 245).

The cnidoblast or nettle cell is found in the basal layer of the epidermis in *Hydra*, and when young it bears no trace of its future development and function. When called upon to develop *nematocysts*, or stinging sacs, by the need of them on the surface, it produces them in its distal cytoplasm which is enlarged and drawn somewhat toward the surface at this time.

The nematocyst first appears as a small, rounded mass of greater density than the surrounding cytoplasm and, as it enlarges, it develops a less dense interior. Its distal wall is invaginated into this interior as a hollow thread which, when developed, lies coiled in the space. The external opening of the lumen of this invaginated *tube thread* is closed by a thin cover. The interior of the sac-like portion or *capsule* is filled with the secretion which is a fluid in the ripe organ.

The mature capsule (Fig. 343) with its contained parts does not lie

primarily in the cytoplasm, but in a vacuole space which is bounded by another distinct membrane, the *vacuole membrane*. The walls of this membrane are continued from its sides down to the lower part of the cell by a separate wall of membrane. Between the capsule and the vacuole membrane lies a very small amount of fluid in the mature cnidoblast.

One other important organ is developed in the cytoplasm in connection with the nematocyst. This is a pointed, chitinous rod called the *cnidocil*. This rod projects distally beyond the surface of the surrounding epithelium, and, when touched, it acts so as to stimulate the cell, contract the capsule, and force the thread to evert. This eversion is a very efficient method of causing the thread to penetrate the body surface of an enemy. Spines are placed on the inside of the folded or invaginated thread so that when it is everted they will come out, point forward, and penetrate a hard body first, thus making a way for the softer thread to follow. Thus, the method of eversion does not involve any traction or friction, which the thread is too delicate to bear. Once in the victim's body, the spines are thrown backward and serve to retain the thread. In passing into the victim the thread carries a poison with it.

A very different type of integumental organ of offense is found in some of the Echinoderms, the sea-urchins or *Echinoidea*. In these animals the body is covered with spines which project from the surface and are movable on a knob-like process of one of the plates with which this animal's skin is provided. In some forms the spines are comparatively harmless, but **the spines of a very common, black urchin**, *Diadema*, found on all tropical coasts, are of tremendous length and exceedingly sharp.

Such a spine consists of a core of mesodermal origin and an outer integumental layer. This outer layer originally has an epithelium continuous with the rest of the body, but when the spine is mature this epithelium is rubbed off, leaving the hard, cortical layer of lime for an outside covering. Near the base, the epithelium persists and, in *Diadema*, is subject to interesting modifications (see Chapter XIII, on Eyes).

The internal part of a spine of *Diadema* (Fig. 344) consists of a long, central canal for circulation surrounded by a mesodermal tissue covered with epidermis. The tissue about the canal is built out into a number of radial plates which are attached longitudinally to the canal-bearing core. These plates meet folds of the integument which project inward and thus form a series of tubular spaces in which the lime tissue of the spine is laid down. At first this lime is an almost solid rod, but as the spine grows, a connective-tissue reticulum is formed and acts as a basis for the additions of lime as shown in Figure 344 at *cal*.

The base of the spine is flexible, owing to a disk-shaped region in which no lime is deposited, and where the connective tissue is developed

centrally into a ligament. The peripheral mesodermic tissue of this same region forms a circular sheet of longitudinal muscle fibers which, by pulling on one side or on the other, is able to move the spine in any desired direction. Around the base of most echinoderm spines is a ring of dermal tissue in which nerve cells and their fibers cause a considerable thickening. This is cut tangentially in the figure.

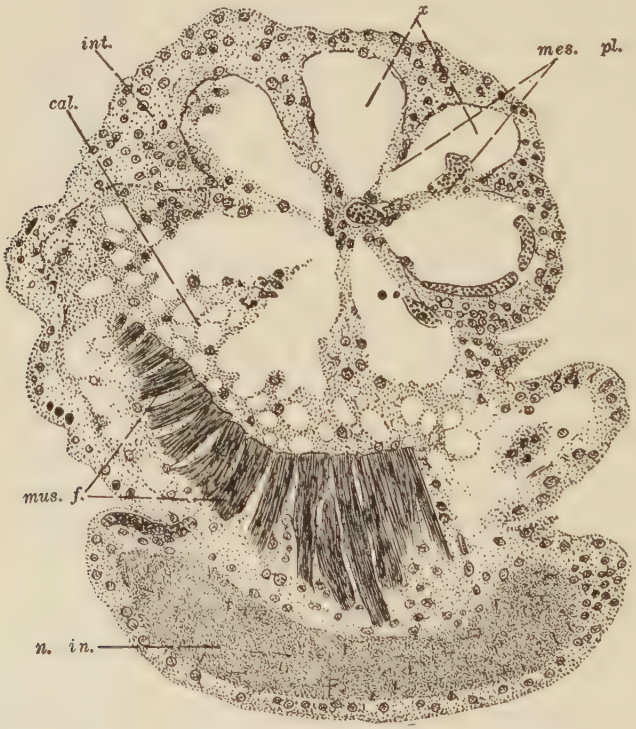

FIG. 344. — Section through the base of a poison spine of the echinoderm, *Diadema setosum*. *mes.pl.*, mesodermal plates radiating from the central canal (here occupied by one of the amœboid blood cells); *x*, spaces occupied by the youngest plates of lime; *cal.*, organic base of the older calcareous tissue; *int.*, outer integument; *mus.f.*, some of the muscle fibers which move the spine; *n.in.*, integument containing the mass of nerve tissue. × 520.

The spine makes a painful wound, and its outer tissues probably secrete an irritating fluid. **In another form**, *Æsthenosoma*, the tip of the spine is enormously enlarged and its upper point invaginated into a pocket. From the base of this pocket arises a spicule which is a continuation of the core of the spine and the whole sac is filled with a poisonous fluid secreted by the lining cells (Fig. 345). When the delicate head of the spine is touched by any creature, the top breaks or caves in, and the spicule is forced into the victim's flesh. At the same time the muscles

of the enlarged head force the poison out into the wounds, causing much pain in a man, and death to some smaller animals.

The insects have developed formidable weapons by which they kill their prey and injure their larger enemies. The best known examples are the bees and mosquitoes. **The apparatus in the butcher bee (or ground hornet)**, *Scolia dubia*, **consists of a cuticular formation, the sting**, which is made up of several parts and worked by special muscles and nerves, **and a poison gland** with its reservoir, which is an invaginated portion of an internal integument, if the lower part of the intestine can be so called. As the poison gland is, perhaps, the more important from our point of view, we shall devote our time to that tissue, merely remarking that the several parts of the sting and its sheath are elongated processes of chitin formed by long, hypodermal cells.

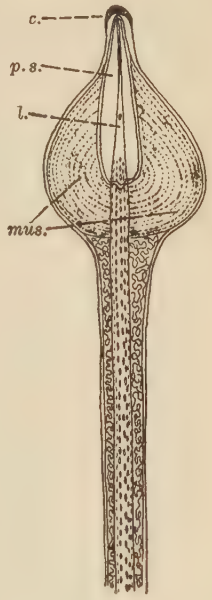

FIG. 345.—Tip of the spine of the sea-urchin *Æsthenosoma*. *p.s.*, poison sac; *l.*, lance to puncture enemy; *mus.*, muscular cushion to squeeze out poison; *c.*, epithelial cap which is ruptured when the spine is used. (After P. and F. SARASIN.)

The duct and reservoir are lined by a very delicate hypodermis, covered by a thin and flexible chitin. The real poison gland is a tubular invagination lined with a cuticle whose walls are invaginated into many fine tubes. These tubes extend proximally into a series of elongated columnar gland-cells in whose cytoplasm they pursue a short course and end with a cylindrical enlargement which appears to act as a physiological filter (Fig. 346). The poisonous secretion is conducted by these tubes to the sting, from which it runs in fine streams that emerge behind the barbs. A layer of thin cells with very small nuclei extends between the gland-cells and the cuticle. Their function and origin must be the same as in the odorous gland of *Belostoma*, which see.

Among some insects, particularly the larvæ of Lepidoptera are found some poisonous spines which are very efficient in their structure and operation. They are specializations of the insect hair, which can be modified to form structures for so many other purposes.

In the case that we shall select for examination, *Sibine stimulea*, **the hairs are compound structures**, extending from several wart-like projections on the sides of the body, and particularly from four large horn-like processes, two of which arise on the head and two on the tail of the larva. The particular hair that we are examining is represented by Figure 347, which shows an oblique section of the basal part at the point where

it emerges from the thick cuticle of the horn-like process. This cuticle on the horn is very thick indeed and is lined internally by a well-developed hypodermis, inside of which is some connective tissue and a few blood cells and other cells. Where a poison hair takes its origin the hypodermis is evaginated to form a cylindrical layer extending obliquely up through the cuticle and finally emerging at the surface as a hair-like structure covered with its own thinner cuticle which is, of course, continuous with the thick layer. This whole structure is extended within the entire length of the hair, which is closed on the end except for a number of fine openings through which the poison can pass out.

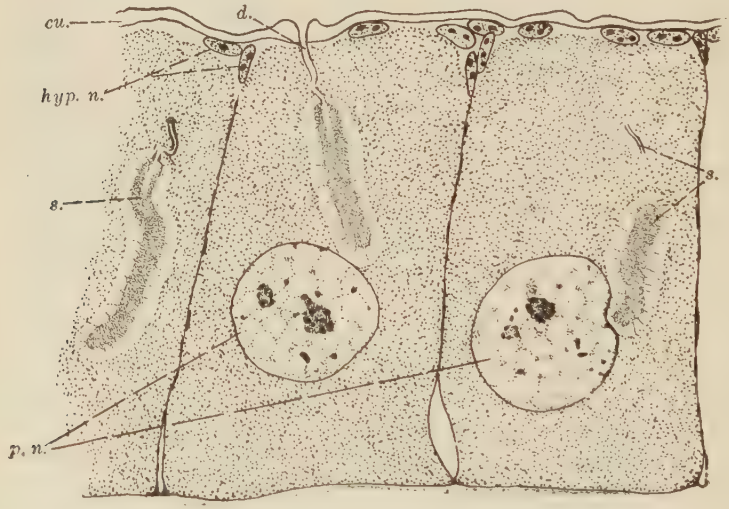

FIG. 346. — Three poison cells from the epithelium lining the poison gland of the ground hornet, *Scolia dubia*. *p.n.*, poison cell nuclei; *cu.*, cuticle on distal surface; *hyp.n.*, nuclei of the hypodermal cells that form the cuticle. At *d.* is the duct-like invagination of the cuticle which ends in the poison sieve or secretion sieve at *s*. Parts of others may be seen in the other cells. × 1200.

The poison is produced by a large cell lying inside the hair and its hypodermis, at about the level of the hair's emergence from the horn. It is a very large and thick cell, with a large branched nucleus, and it often looks to be, and may be, a syncytium. Its large cytoplasmic body is hollow at its distal part, and this hollow end is produced into a tube which carries the poison up into the hair inside of the hypodermis.

The presence of openings in the hair to permit the poison to escape has been questioned or even denied by some writers. The present writers have not been able to see them and, therefore, think that the great irritation (which has certainly been felt) is due to the breaking off of the spines in the flesh, and the consequent introduction of the poison. No

muscular apparatus has been found that could be used to inject the venom.

The Arachnids show a rather remarkable histological advance on the usual insect types in regard to their poison glands. In place of the very

FIG. 347. — Slightly oblique section through the base of a poison hair of *Sibine stimulea* where it leaves the skin. *p.c.*, poison cell (a syncytium) with a hollow lumen to carry the poison outward. *hyp.c.*, hypodermal cells which make the hair cuticle. × 435.

remarkable secreting cells, with the cuticle that lines the usual form of insect poison gland, we find a gland whose secreting cells are naked distally and which possess no peculiar intracellular differentiations.

The poison glands of the spider, *Lycosa sp.*, form a good example. These glands are integumental invaginations from the tips of the biting mandibles, and they extend into the anterior part of the thorax.

A transverse section of this tubular gland shows the following main layers. A single outer layer of large muscle fibers which extend the length of the gland and are consequently seen in transection. The sections are roughly square or triangular owing to the compact way in which the fibers are arranged in the layer. The illustration (Fig. 348) shows three of these structures. Their myo-fibrils are placed in radiating plates or muscle columns, and the free sarcoplasm is collected in the center where the nuclei are also most frequently found.

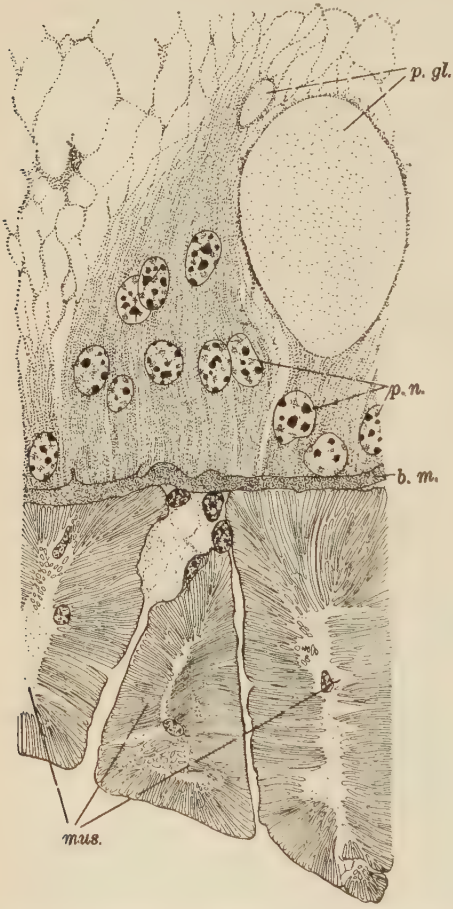

FIG. 348. — Section of the wall of the poison gland of a spider, *Lycosa speciosum*, seen in cross section. *mus.*, three muscle cells; *b.m.*, basement membrane; *p.n.*, poison-cell nuclei; *p.gl.* globules of poison. × 580.

The next layer is formed by a basement membrane which is homogeneous and much thicker than such structures usually are. The poison epithelium is probably the active agent in its formation.

Inside of the basement membrane is found the poison-secreting epithelium. This layer is composed of a single layer of very high, narrow cells whose lateral boundaries are difficult to see. The nuclei are large and placed well up from the basement membrane, although they are irregular in that respect and some of them rest close to it. These nuclei have a peculiar chromatin pattern which close study might enable one to specifically identify from the other nuclei of the animal. The cytoplasm is most markedly striated by fibrillar structures, which reach from the basement membrane to the distal edge of the cell. In fact they reach beyond this edge, and so obscure it that it cannot be definitely determined. Outside of the cells these fibrils end in a loose reticulum which fills the lumen and contains the poisonous fluid in its meshes.

The poison appears as a few yellow, scattered droplets in the outer

cytoplasm of the cell. These grow into very large drops which seem never to blend, but to work their way individually down through the reticulum in the lumen. Comparatively little of this matter is secreted and it is possibly only a part of the material which goes to make up the poison used by this creature to kill its prey, the rest being a soluble fluid that does not appear in the usual microscopic preparations.

These cells extend into the duct of the gland as low, non-secreting, epithelial elements, thus proving the origin of the poison cells. The poison cells of the scorpion and allied forms appear in the tail and other parts of the body, but have much the same histological and cytological structure as those we have just examined.

The vertebrate animals are not without their poison-producing members, and some of these are among the most dangerous creatures known, on account of their size and the amount of venom that they are able to inject into a wound. These animals also have the mechanical structures of offensive protection remarkably well developed.

Among the fishes some spines, which are mesodermal bony structures, are developed in connection with the fins. Most of these are not poisonous except for the slime and dirt that are associated with them. The **sting of the whip ray**, *Dysatis*, and other sting rays, makes a very ugly wound. It is barbed and when broken it is renewed from a tissue nucleus that forms new spines, slowly, all the time.

Some fishes also have the first ray of the two pectoral and the dorsal fins enlarged and barbed to use as a weapon of defense. **In some catfishes, as *Schilbeodes*, the pectoral spine is associated with a weak poison gland** (Fig. 349, *A* and *B*), which appears in two forms; an axillary gland opening by a pore near the origin of the fin in most of the species, and a glandular tissue placed between the skin and spine in those catfishes which do not have barbs on the spines.

The axillary gland is clearly an invagination of the stratified surface epithelium whose cells are specialized to secrete poison. They proliferate and swell up until they finally burst, and the poison is discharged from the pore. As can be seen in the figure, these poison cells are modified clavate cells which occur in all the skin of this and other fishes. One needs but to trace the row of clavate cells (Fig. 349, *A* and *B*, *c.c.*) from the outer epithelium around and into the poison gland to realize this fact.

In the fin spines of such of the catfishes as have no serrations on these spines, may be seen another collection of the same poison cells. Here they lie between the integument and the spine. No duct is apparent, and we must examine a longitudinal section of the tip of the spine before the relations of the poison cells to the epidermis can be understood.

Such a section (Fig. 349, *A*) shows that the epithelium on the end of the spine has been reflected as a blind invagination around the central

bony spine (*sp.*) and that the poison cells are developments of the clavate cells which were carried in with this epithelium. In fact, the one kind can be continuously traced into the other in Figure 349, *A*.

Such tissues are also found in many other bony fishes. The "weavers" and their allies have them, and in *Thalassophryne*, a marine form, they are developed to formidable proportions.

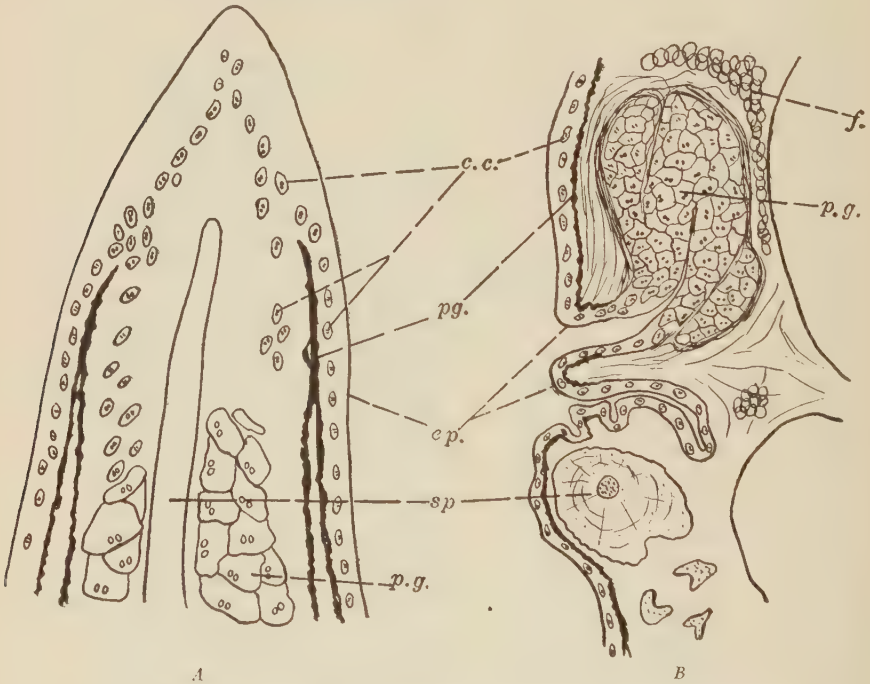

FIG. 349. — *A*, tip of a fin-spine of the river catfish, *Schilbeodes; B*, section through the body wall to include the auxiliary poison gland and the base of the pectoral spine of this fish. *ep.*, epidermis with clavate cells (*c.c.*); *p.g.*, invaginated epidermis with clavate cells modified to become poison cells; *sp.*, spine; *pg.*, pigment cells; *f.*, fat cells. Both clavate and poison cells have double nuclei. (After H. D. REED.)

Passing by the amphibians, whose so-called poison glands are used mostly to produce offensive odors, we find that the reptiles have the best-developed venomous organs. **In the rattlesnake**, *Crotalis horridus*, a beautifully developed tooth is transformed into a fang by the presence of a groove on its anterior surface. This groove is turned into a tube by the overlapping of its edges, leaving an upper and a lower aperture.

Above, in the connective tissue on the side of the jaw, an invaginated region of the integument forms a large gland with many irregular, alveolar lobules each of which is lined with a simple cuboidal epithelium (Fig. 350, *A*, *B*). The cells have a large, somewhat flattened nucleus

and show no vacuoles, granules, or other marked signs of secretory activity (Fig. 350, *B*). They secrete the poison, which fills the large cavities of the gland and is sent through a duct to the upper opening in the fang. When the snake strikes, a powerful muscle compresses the gland and forces the venom out of the fang in a jet. The fangs are constantly being formed as teeth are, and several half-developed ones are always to be found in the gum, ready to come out and take the place of the old one when it is lost.

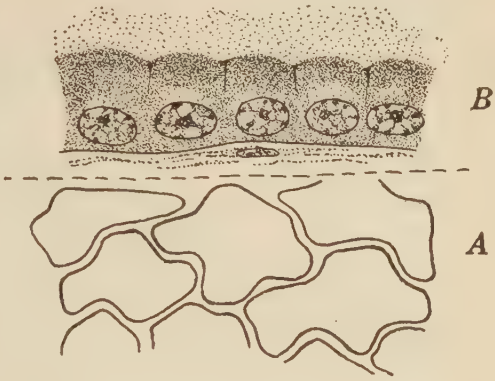

FIG. 350.—*A*, outline of several lobules of the poison gland of a rattlesnake, *Crotalis horridus*, as they appear in a section of the gland; *B*, five cells from the epithelium of these lobules, enlarged to show their finer structure. The colloid substance distad of the cells is the poison. × 1500.

Another reptile, the Gila monster, has poison glands developed in the integument of the mouth near the lower teeth. A bite from this creature is only venomous when it succeeds in turning on its back and thus draining the poison into the wound.

The birds and mammals produce no poisons but have many of the mechanical integumentary forms of defense and offense. Among these may be merely mentioned teeth, claws, spikes, spurs, spines, two distinct kinds of horns on top of the head, and one on the nose, to mention but a few of them. We shall briefly describe but two of these structures, **the claw as exemplified by the human nail**, and **the spine of the porcupine.**

The nail is a cornified outer layer of stratified epithelial cells, developed in a folded area of the skin on the ends of the fingers. Two regions of this fold are distinguished; one, by the fact that the lower posterior body of the nail is formed there and grows forward out of it, and the other by the fact that the nail is not materially added to as it grows past this region. Both these regions are on the under side of the fold, and are known as the *nail bed* (Fig. 351).

The other side of the fold also rests against the nail, on its upper surface, but this epithelium does not contribute in any way to the nail's formation other than to protect it from drying during the weak beginnings of its growth. This surface is widest over the proximal "root" of the nail, and forms overlapping ledges on each side. Its epidermal layers are practically the same as on the rest of the skin.

The basement membrane of the nail bed is thrown into longitudinal

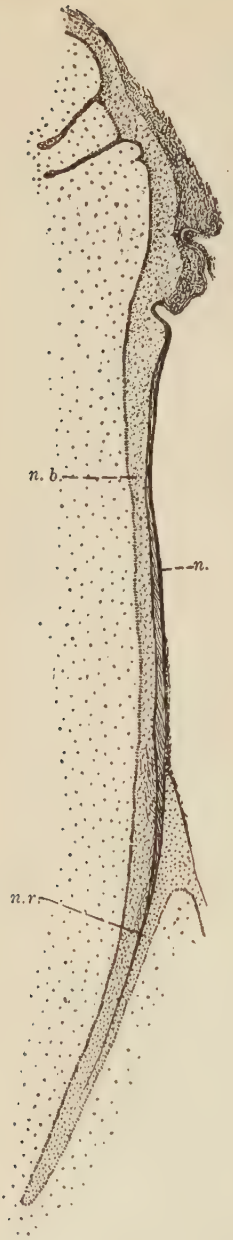

FIG. 351.—Longitudinal, vertical section of the young nail and nail bed of an infant. *n.r.*, nail root or lunula; *n.*, nail; *n.b.*, nail bed. (From a preparation by DR. H. E. JORDAN.)

furrows, and the layer homologous to the *stratum Malpighi* is developed from the basal layer. It fills the furrows and covers the ridges, thus forming a nearly level surface. From this surface these cells are added to the nail above, in such a manner that they overlap each other like roof tiles. They become hardened and transparent.

Claws are formed much as are nails, also some forms of horn, as the cow's horn. The horn of the rhinoceros is more to be compared to a collection of jointly formed hairs of great size and strength. The spurs on the wing and shank of birds have a bony core as does the cow's horn.

Even hairs can be specialized into a set of very formidable weapons. In the porcupine, *Erinaceus*, they are immensely long and strong, and have pointed and barbed ends which are very injurious to their victims. On account of its size this hair is developed by an exterior or lateral hardening of the superficial layers of stratified epithelium on a deep-set papilla (Fig. 352). In this respect they resemble a feather somewhat, especially as the papillar epithelium is thrown into longitudinal ridges to strengthen the shaft and form the distal barbs.

Technic. — The cutting of sections of the materials mentioned in this chapter is often very difficult owing to the extreme hardness and toughness of some of them. Certain small or delicate hairs, feathers, and nails may be ignored and sections cut as usual after any good fixation in the usual fluids. With the larger and harder kinds, the best general

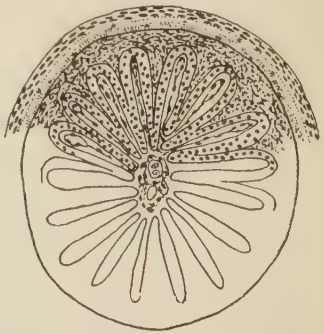

FIG. 352. — Transverse section of the developing spine of a porcupine, *Erinaceus*. (After DAVIES.)

aim is to fix in some fluid that will harden the elements as little as possible and then try to embed the tissue at once and as quickly as is possible. Under favorable circumstances this may result in the least amount of hardening, and, if the knife is very sharp, fairly good sections may be obtained.

If the horny matter is exceedingly refractory it is better not to try to cut it *in situ*, but to separate it from the surrounding tissues, and cut them separately, the soft parts in the ordinary way, and the horn or nail after some of the macerating and softening methods such as 40 per cent potash solution or strong mineral acids or javelle water.

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INTEGUMENT, LUBRICATION

The bodies of most organisms are lubricated with some fluid, and this fluid also acts in other capacities in many cases; as a preservative, a cleanser, a food-gatherer, or even, deserting its original duty, it may be developed as a poisonous substance or a foul-smelling or attractive-smelling material accordingly as it best serves a purpose. Sometimes the fluid is used to lubricate some particular portion of the body-surface or it may be found on the entire surface.

The fluid may be produced from all parts of the body-surface or by some parts of it only, which may be further defined from the rest by being invaginated into glands that pour out their secretions on such parts of the integument as require it. We may distinguish, according to the kind of fluid that is produced, three principal kinds of lubrication tissues.

1. A tissue that produces *a slimy material called mucin.* This substance has a definite chemical basis and is a product that can be used for other internal processes or external processes than those of lubrication, such as preservation of the integument by the retention of water, cleansing, the collecting of food particles, the making of cocoons for the eggs,

and the making of dwelling cavities for the entire animal. The specific cells of this tissue are not to be confounded with those of the serous tissues which produce their secretion in a similar way. This method of lubrication is mostly characteristic of animals that live in the water although many of these spend large periods of their lives in the air. Here, however, they must be in a damp location, for it is true of mucus that it will not withstand a thorough drying.

2. A tissue that secretes *an oily substance called the sebaceous fluid*, whose primary use is to lubricate the skin and its appendages, and to preserve them from the effects of drying. Among its principal secondary uses is the production of odors.

This tissue is found in those animals that live in the air, although it has persisted in some of those that have later adapted themselves to the water. The only alternative open to an animal that lives exposed to the drying effects of the air and sun is to have a hard, impervious covering of cuticle as in some of the Insecta, Crustacea, and lower vertebrates. These animals dry very rapidly when confined without a supply of water, showing that their cuticle does not entirely protect them.

3. A tissue that furnishes *a "serous" secretion for lubrication purposes*. This form is somewhat rare, and is usually found in parts where the surface to be lubricated is internal, as in the joints between the bones, or semi-internal, as on the surface of the eye. In this last case the effects of drying are avoided by the frequent renewal of the serous fluid by the act of "winking" or rapid closing of the eyelid, thus carrying the fluids over the surface of the eyeball.

These fluids are of several kinds, and originate in several different secretory surfaces or in glands that communicate with these surfaces. Some lubricating glands are distributed over certain surfaces of the mammalian body and are called the sweat glands. Others are confined to particular regions and perform very special duties as the wax glands of the ear tube. To cover the field thus laid out we shall examine the following cases.

1*st*, a general lubrication of the body of a water animal with mucus, accompanied with the removal of dirt and collection of food: the clam, *Mya arenaria*.

2*d*, the production of mucin by a land snail, *Mesodon*, from cells of extreme specialization.

3*d*, a general and complete lubrication of the body, and the animal's habitat with mucus: the earthworm, *Lumbricus*.

4*th*, a general lubrication of the body-surface of an animal living in the air, with mucus (mixed with a poisonous or offensive substance): the toad, *Bufo Americanus*.

5*th*, lubrication of parts of the body with an oil produced by a gland: the sebaceous glands of the cat and the oil glands of the fowl.

6*th*, the lubrication of the eye in the alligator and mouse.

7*th*, the lubrication of the joints of a cat's bones by the synovial fluid.

8*th*, the sweat glands and wax glands of the mammals.

The Mucous Tissues of the Clam, *Mya arenaria*. — If the mantle-fold of a living example of this mollusk be drawn apart and the surface of the inner sides of the mantle as well as the surface of the foot be examined, it will be noticed that bits of paper, particles of dust, and any other small objects that may be dropped on this surface will move along, always in a definite direction or path. A mapping out of all the paths so determined will show that they form larger courses, of various curves and straight runs, all tending toward and uniting to end at the labial palps, and then passing from these to the mouth.

A section of one of these regions (Fig. 353) will show two facts; the presence of many mucous cells among a vastly greater field of ciliated cells which, in life, are always moving their cilia at any one point in the same direction, and that direction the same as one of the paths. Thus, it is the cilia that furnish the motive power, but the food and the dirt particles would not cling to the cilia alone especially under water. Here the mucous cells of the integument play their part by providing a thin viscid covering to the ciliated surface. To this sticky surface the particles of food as well as the particles of sand, dirt, etc., in the water stick and the whole mass is thus carried along and finally engulfed in the mouth. The lubrication cells (which here produce mucus) do comparatively little of what we might strictly call lubrication. This is especially true of some of the fixed plecopods, as the oyster, in which there is no active foot and the animal scarcely moves in the quiet recess of its shell. In other forms that lead an active life, especially the kinds that burrow rapidly in the sand, as *Unio*, *Mactra*, or *Ensatella*, there is a considerable amount of contact and abrasion between the strong, muscular foot, the mantle and other parts, and here the mucus acts as a viscid, sliding buffer between the delicate surfaces. In other words it acts as a true lubricant. *Mya* is a form that, so far as motion and a consequent lubrication is concerned, occupies a position about midway between the two.

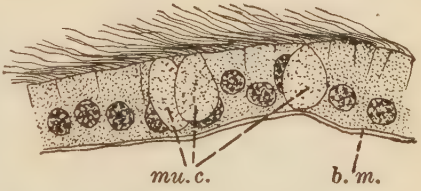

FIG. 353. — Body epithelium of the clam, *Mya*. The majority of the cells bear the numerous cilia on their distal surface. *b.m.*, basement membrane; *mu.c.*, mucous cells with round bodies and flat, crescentic nuclei.

The mucous cells lie singly in different parts of the epithelium and

are much more abundant in some parts than in others. They usually are cells of about the same length as the surrounding ciliated cells although they are by far wider owing to the distended cell contents. This latter consists of mucin granules which fill the entire cell, packed full, and gives it the round shape.

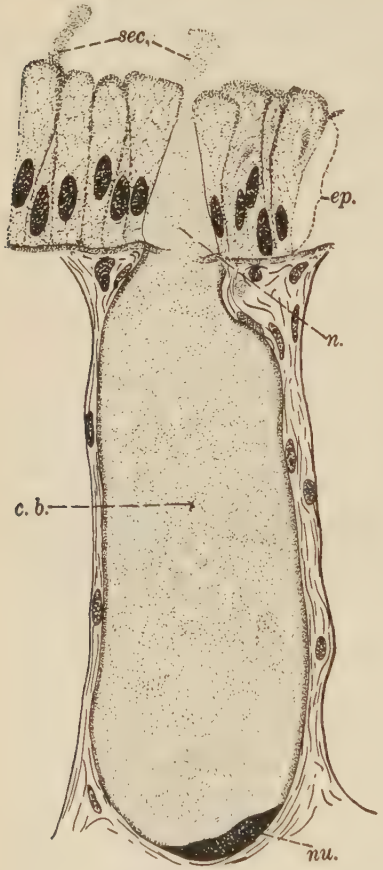

FIG. 354. — A deep mucous cell from the mantle edge of a land snail. *c.b.*, large cell body filled with secretion; *n.*, neck through which secretion is discharging; *sec.*, secretion of this and another similar cell to left; *nu.*, nucleus; *ep.*, surface epithelium to which this cell belongs morphologically. × 1000.

The nucleus is crowded to the cell-wall and becomes much flattened. It lies against the wall, but in this particular case it may as often lie against the side wall as against the bottom, where it usually lies in most other mucous cells. The nuclear content is very much compressed. The mucous cells bear no cilia, and their secretion is discharged as a swelling mass of mucus of thread-like form. As to the periodicity of this discharge or the renewal of the cells no facts were observed.

Some of these cells are so large, in this and other mollusks, that they cannot be contained in the epithelium. In this case the proximal part of the cell grows or pushes down and lies in the connective tissue beneath the epithelium.

Many of the **mucous cells of a land snail**, *Mesodon tridentata*, show this specialization at its extreme, and in Figure 354 one of these cells and the sides of two others are pictured to show their structure and relations to the ordinary epithelium cells between which they open. As in all mucous cells, the nucleus lies flat against the cell-wall and in this case against the bottom of the cell. It is large and its chromatin is compacted so that its particles form an almost solid mass.

This principle is carried one step further in the nidamental cells of the leech, an account of which will be found in Chapter XXII, and should be glanced over in this connection.

Mucous Lubricating Cells of the Epidermis of the Earthworm. — The slime cells are well represented by the mucin-producing cell of the epidermis of the earthworm. These cells may be considered as certain of the simple epithelium cells that cover the surface of the body, and that have been differentiated in structure so that they are able to secrete the specific substance called mucin. The mucin is produced as good-sized, hard granules in the cytoplasm of the cell, and has the property of mixing with water into a jelly-like, viscid mass of many times its original size. The substance can be identified easily in a number of ways by its staining properties and by its solution reactions in different media. The granules of mucin are produced in the cytoplasm of the cell and in regions of this cytoplasm that are arranged in more or less straight rows reaching from the proximal to the distal end of the cell. The substance of the mucin comes from the blood as a fluid, invisible to the observer, and this fluid is converted into the mucin by the activities of the cytoplasm in ways that we cannot as yet understand. They appear as granules of a small size and grow to their full size, which is considerable (Fig. 355). When fully formed the granules fill the entire cell to such an extent that it is distended to many times its original bulk. The reason of this great distention is that the cell matures all of the mucin granules at once. This is not true of some other mucin-producing cells which contain the granules in all stages of maturity, and which are constantly giving off some that are ripe. No matter how distended the cell may become it never moves proximally out of the line. When the earthworm's cell is ripe it discharges its contents in a very short time and is then seen in a state of collapse. Some peculiar differentiated areas in the cytoplasm now show the positions that were occupied by the granules, and the nucleus, instead of being crowded down against the bottom of the cell and flattened out, has arisen to a point about one fourth of the height of the cell and has assumed a round contour and the characteristic chromatin and nucleolar conditions of functional activity. The cell begins, after a very short rest, to secrete mucin again.

FIG. 355. — Vertical section of a bit of epidermis of the earthworm. Shows four mucous cells in different stages of secretion, mostly later stages. *cu.*, cuticle with two pores shown, from one of which mucus is emerging. × 1100.

The mucin is extruded from the cell through the fine pore in the

cuticle that is found opposite each gland-cell. It is used not only to lubricate the body but to permanently line the tube in which the animal lives with a mucous covering.

These cells are, in some stages, very much like a number of the other epidermal cells in the epithelium of the earthworm that are also modified for the purpose of secretion. This other kind of cell, however, produces a very different kind of secretion, and it might well be studied as a type of the so-called serous cell or albumen cells of the animal tissues. It differs in the earthworm from the mucin cells in several ways. Most important is the staining reaction of the secretion. This and chemical tests show that it is a different substance from mucin although it is possible, considering the derivation of the two cells and the similarity of the manner in which they secrete, that both cells and secretions were derived from a common kind or that one was derived from the other.

The simple, unicellular, mucous gland may thus be followed through a series of forms of increasing specialization terminating with the extreme form found in the leech which is discussed in another connection (Chapter XXII). This single mucous cell is found, in some very rare cases, in an epithelium that has become stratified, and Figure 356 shows a case of this kind in a section of part of the **alligator's conjunctiva**. Here a single mucous cell is to be seen rising from the basement membrane through the several stratified layers, and opening freely to discharge its secretions. Similar conditions are found in the skin of many fishes.

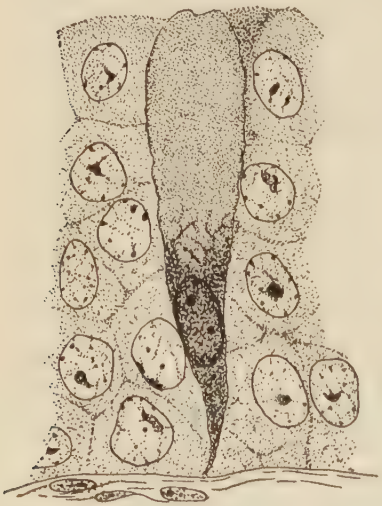

FIG. 356. — Vertical section through a bit of the stratified epithelium lining the alligator's conjunctiva. Shows one mucous cell extending through the epithelium. × 880.

The mucous cell is found not only in the single-celled forms dealt with in the above paragraphs, but also collected into groups that are mostly invaginated into glands of varying complexity. Such a gland may be found to be derived from, and opening on to, a simple epithelium or a stratified epithelium. The former should be considered a more primitive form, perhaps, and can be easily seen in the mucous secreting glands which open into the small intestine. These are known as the *duodenal glands* or *Brunner's glands*, and the simple epithelium which lines them can be traced through their ducts and into the simple columnar epithe-

lium that lines the whole intestine. Another example, which does not so well illustrate the principle, is seen in the sac-shaped mucous glands which open out on to, and are derived from, the olfactory epithelium of the nasal cavity. This epithelium is hardly simple, however, and a still simpler and better example is seen in the many tubular mucous glands that open into the lumen of the uterus of many mammals, as the cat.

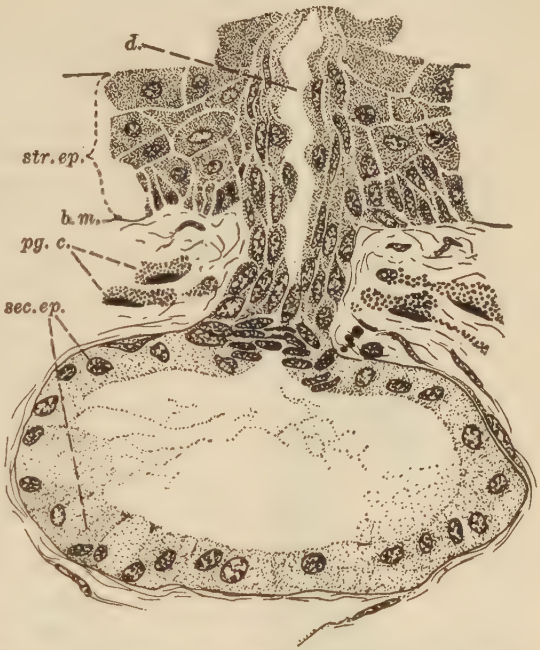

FIG. 357. — A many-celled, succular, mucous gland from the skin of a toad. *str.ep.*, stratified epithelium on surface; *sec.ep.*, secreting epithelium in gland; *b.m.*, basement membrane; *d.*, duct; *pg.c.*, pigment cells. × 520.

Of mucous glands which are derived from a stratified epithelium we have many examples, and such glands may be noticed in the mucous glands of the Amphibia. These are each composed of a few typical mucous cells that are in communication with the exterior by means of a short duct that is also, of course, constructed of the epithelial cells. In many cases the duct is constructed of a single cell, and the cells immediately connecting it with the body of the gland are specialized to control the flow of the secretion. When the epidermis is shed in the periodical molts of the animal, the cell or cells that form the duct are shed with it. This form of gland is well shown in Figure 357 from the toad. The gland-cells in this and the following case appear to represent the basal layer of the stratified epithelium from which the gland was developed.

Such a simple form of invaginated gland is also represented in the higher forms (mammals) by the mucous glands found in the posterior portion of the tongue. Here a large number of glands like those found in the salamander are joined together and empty the secretion out through a common duct that has many branches. Still larger collections of the same structures form some of the large, salivary glands that lie far back

between the muscles and the integument on the side of the head, where they form important anatomical features. See the chapter on alimentary tissues of the animals in question (see Chapter XV).

Sebaceous or Oil-Lubricating Tissues. — The second class of lubricating tissues is found almost exclusively in the integument of the higher vertebrates (mammals and birds), where there is developed a type of gland that is used to produce a fluid for lubrication. This type is essentially different from the mucous type in most important respects. It produces an oil instead of mucin. The cell is nearly always destroyed by the act of secretion instead of repeating its duty a number of times. Also the cells that do this always occur in a stratified epithelium in which they can be more conveniently renewed than in a simple layer of cells. The cells that do this work never operate alone but always in some considerable extent of epithelium which is usually invaginated to form a gland. This form of tissue is sometimes secondarily adapted to produce an oil that is attractively or offensively odorous (see next part). It is used most commonly to lubricate the hair in mammals, and the feathers in the birds, and our first study may very properly be on the common sebaceous gland of the cat's skin.

FIG. 358. — Root of a small hair in the lip of a cat to show the sebaceous gland (*seb.gl.*); *sh.*, shaft of hair; *n.*, neck or duct of gland in which the secretion collects. (Taken from a preparation by DR. H. E. JORDAN.)

Sebaceous or Lubricating Gland of Mammals; from the Lip of a Cat (Fig. 358). — These glands are found clustered around the hairs at the point where they are about to leave the skin and where the sheath is plainly distinguishable as an invaginated continuation of the epidermis. Being glands that are invaginated from this sheath, we may say that they are invaginations of the stratified epithelium.

The stratified epithelium is carried into the invagination, but is so

much thickened inside, that it practically fills it up and leaves very little lumen to be seen. This gives the gland a solid appearance with the basal cells of the stratified epithelium at the fundus. These basal cells appear to be in the same condition that they exist in other parts, but the distal layers become larger as they approach the surface of the lumen or, if there is no lumen, the neck of the gland. Knowing that stratified epithelium proliferates from the basal layer, we can follow the history of these cells of the sebaceous glands and see that, after a cell has been divided off from the basal layer and started on its journey towards the surface, it undergoes changes that are very different from the well-known changes seen in other and unspecialized parts of the outer epithelium of man.

Instead of forming keratin it goes through a process of vacuolization in which the vacuoles are filled with the oil which has been elaborated at the expense of, and through the destruction of, the cytoplasm. As the elaboration of oil continues, the nucleus disintegrates so that the production of oil in these glands results in the destruction of the cell.

The oil drops begin to appear in the part of the cytoplasm next the nucleus, as numerous and small vacuoles. They rapidly increase in size until they fill the cytoplasm, and by the time that the cell has reached the surface of the epithelium, or neck of the gland, it appears as a mass of oil drops inclosed in a sac, the cell-membrane, and containing the remains of the nucleus. A number of these ripe cells collect at the fundus and rupture to form the glandular discharge. The secretion thus contains the degenerated nuclei and cell-membranes of the cells that produced it and which, we can now see, were completely sacrificed in the production of one portion of the secretion of the gland. This is not the case with most glands.

Sebaceous or Lubricating Glands in the Birds; Oil Glands of the Chicken. — Here are found two large groups of sebaceous glands lying parallel with one another (or nearly so), and all the glands of each group opening into a single cavity, itself an invagination of the integument on the rump of the animal. Each of these glands is long and tubular in shape, with a wide and clearly defined lumen which is of equal width for its entire length. The gland shows no differentiation into the neck and fundus. Its long sides are everywhere lined with an epithelium that is very evidently a stratified epithelium of some ten or twelve irregular layers (Fig. 359). These layers all come from a basal layer that proliferates them exactly as in an ordinary stratified epithelium, with the important exception that no amitotic divisions take place in the cells that are moving to the outer surface.

The first two rows of these cells show no change except, perhaps, an

increase in size. When they have attained to the third row, however, it can be seen that a number of vacuoles form in the cytoplasm and these continue to rapidly grow as the cell moves outward, until at the eighth or tenth row, or layer, the cell is many times larger on account of the increase in the size of the vacuoles, and the nucleus begins to shrivel and to lose its structure.

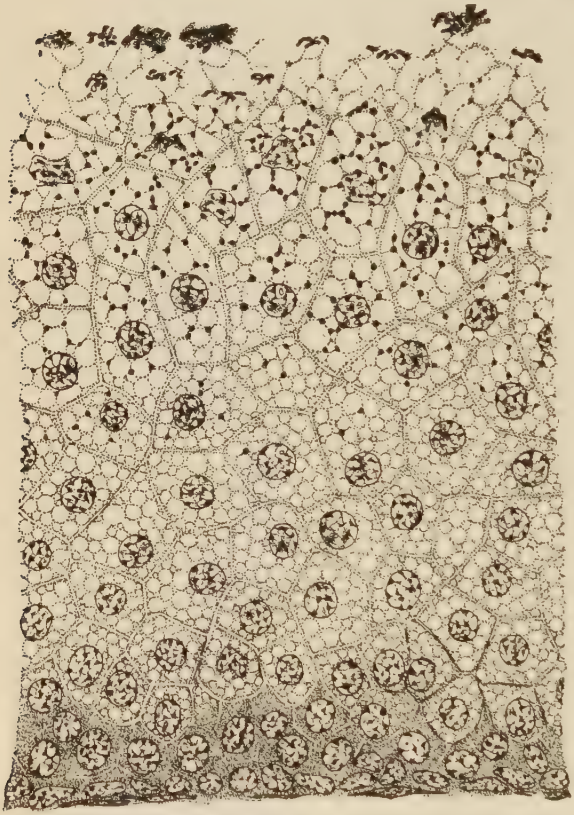

FIG. 359. — A vertical section of a portion of the secreting epithelium that lines the tubular oil glands of a chicken. Basement membrane below, distal surface and lumen above.

The vacuoles are filled with an oil; in the two outer rows the cell structure completely breaks up, leaving only the oil containing some remnants of the nucleus and cytoplasm. The lubricant has been formed, but, unlike the mucous cell, the process cannot be repeated by the cell because it has been destroyed in the process. Several other forms of oil-secreting glands are to be found among the reptiles, birds, and mammals, and some of these have such a secondary use that they are treated of in the next part.

The lubrication of the eye in such vertebrate animals as live in the air is somewhat complex. Two types of gland tissue are employed in this function besides the few true mucous cells that occur in the conjunctiva. The first of these is a tissue that produces a peculiar oily fluid and is represented, in a simple form, by the glandular epithelial surface found on the conjunctiva of the alligator (Fig. 360).

The epithelium is a thin, stratified form, and its layers are but two or three in number in a young animal of eighteen inches in length. It is the outer layer that is interesting because it is not composed of dead

cells as in the surface layer of most stratified epithelia, but of a layer of columnar secreting cells of great activity.

These columnar cells give the epithelium its name "pseudostratified" and, besides their long bodies packed with the secretion product, they are distinguished by having a slightly smaller and more chromatic nucleus which lies in the proximal end of the cell. The secretion of these cells is slowly discharged from the distal surface, and used as a lubricant for the eyelid.

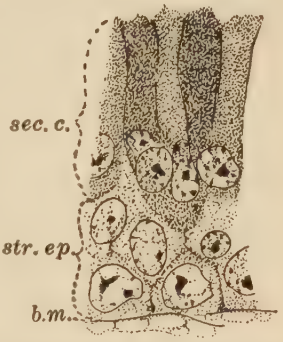

FIG. 360. — Part of the epithelium that lines the conjunctiva of an alligator. *b.m.*, basement membrane; *str.ep.*, stratified epithelium; *sec.c.*, distal layer of secreting cells. × 880.

It will be observed upon examining this epithelium that the secreting cells, unlike those of nearly every other kind of gland, do not lie in close contact with a blood supply. They must, therefore, receive their food supply through the efforts of the cells that lie between them and the blood. This involves unnecessary labor and is rarely seen, the proximal surface of all such cells usually lying directly against the thin wall of an arterial blood supply.

Another form of this kind of lubricating tissue is found in the **tear gland of the mouse.** The secreting epithelium is simple in this case and has been invaginated into a large, compound, saccular gland, a portion of whose secreting epithelium is represented by Figure 361.

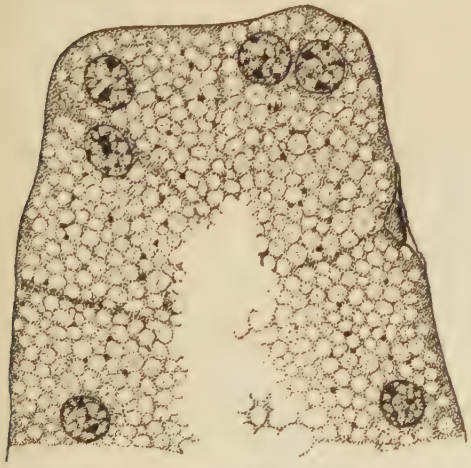

FIG. 361. — Part of a section through an acinus of the tear gland of a mouse. The cells show a vacuolated cytoplasm which discharges its secretion into the lumen. × 800.

At first sight these cells seem to correspond with those of the sebaceous tissue seen in the chicken's rump glands, both as to character of secretion and method of producing it by the sacrifice of a succession of cells. The latter is easily disproved by noting that the cells of the tear gland form a *single* layer, and that there is no evident means of renewal. The idea that the method of production is much the same, however, holds true in

part, in that the secretion appears as little globules, or in vacuoles, in the proximal end of the cell and either moves *through* or *with* the cytoplasm toward the distal end, where it ruptures its bounds and is set free in the lumen, together with a disintegrating portion of the cytoplasm. Notwithstanding this probable cytoplasmic movement, the single nucleus remains in its proximal position in the cell.

The secretion stains black in osmic acid, but is not a real fat because it dissolves in water.

Another form of serous lubrication takes place in the cavity between two bones that form a joint in the vertebrate animals. The membrane which closes in this joint cavity at the sides is known as a synovial membrane, and through its agency is produced the synovial fluid.

The real secretory cells that produce the synovial fluid are not clearly defined from the connective tissues that make up the bulk of this membrane. In larger joints, the membrane is evaginated into the cavity, as a series of short papillæ in some forms, or as a single, septum-like lamella which reaches as far as the danger of being pinched between the bones will permit. **The synovial membrane of the cat** is amplified in this latter fashion, and the illustration, Figure 362, shows a vertical section through a portion of this lamella near its inner boundary.

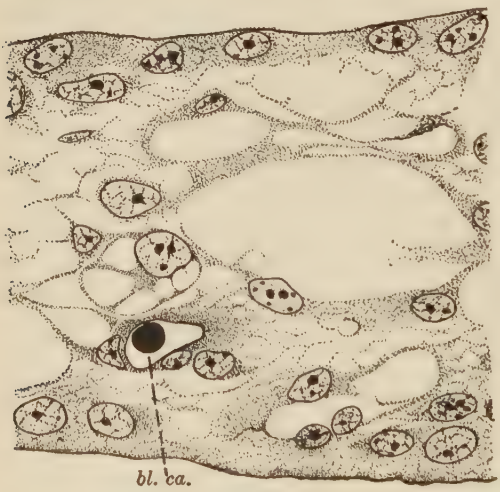

FIG. 362. — Part of a vertical section through the synovial lamella from a cat's joint. *bl.ca.*, blood capillary containing a corpuscle.

The connective tissue that forms the central part of this organ is arranged as a rather wide-meshed reticulum. Blood vessels and lymphatics pass through this reticulum, and from them is derived the water and the very small proportion of other matter which makes up the synovial fluid. The synovial cells that secrete the fluid must exert some specific influence on the selection of its proper constituents. They possess a cytoplasmic body of some size and solidity, and their position on the surface gives them an epithelial arrangement.

The **sweat glands of some mammals** are perhaps to be considered here. While they perform no real function of mechanical lubrication,

they pour out a fluid that is used principally to keep the skin moist and to reduce surface heat by evaporation. These glands are simple tubular glands which are continuous with the basal cells of the stratified epithelium. The duct reaches up through the outer layers of this epithelium to open at the surface.

The gland itself passes down into the connective tissue of the skin and ends as a coil of somewhat similar structure. This structure consists, in the duct, of two or three layers of cells which constitute a weakly stratified layer. The lumen thus formed is bounded by a cuticle belonging to the inner layer of cells. A distinct basal membrane appears between the epithelium and the surrounding connective tissue, and on this are a few longitudinal smooth muscle fibers.

The lower coiled part of the tube is lined by a single layer of cubical or columnar cells which do the active secretion. Several phases of activity may be detected in them, and it is known that, while under ordinary conditions they secrete an oily material to lubricate the skin, when excited by the proper nervous stimuli and blood supply, these glands pour out the watery sweat, probably in addition to the first material.

The sweat glands attain a large size in certain positions, and in some of these larger forms the secretion is probably different. One kind of modification is probably the very large wax glands found in the ear tube. In structure these are very much the same as sweat glands. In function they secrete and discharge a thick oily material that almost solidifies upon contact with the air and serves as a protection to the ear tube against the entrance of harmful insects or foreign particles. Figure 363 shows a section of the epithelium lining the coiled portion of this wax gland with the secretion shown *in situ* in the lumen.

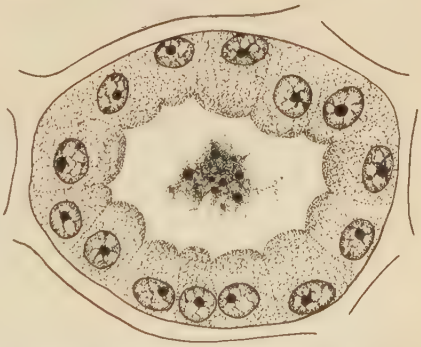

Fig. 363. — Transverse section through one of the secretory coils of a wax gland in the cat's ear-tube. Some secreted material lies in the lumen.

See also the description of the mammary glands in Chapter XXIII, as they are also probably derived from primitive sweat glands.

Technic. — Flemming's fluid and thin paraffin sections will give the best results with nearly all of these tissues. The oily secretions are sometimes stained black, and sometimes not, by the osmic acid. When the lubricating secretion is a *mucin*, it is best to use sublimate or some similar fixative, and then to stain with one of the dyes that is specific for this substance. Most beautiful differentia-

tions may often be obtained in this way. Mayer's muci-carmine is a good stain for this purpose. Delafield's hæmatoxylin is perhaps the best.

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TISSUES FOR PRODUCING ATTRACTIVE AND OFFENSIVE ODORS

In the mammals, and even in the Sauropsida, there are glands of the sebaceous type that are developed and specialized to perform other functions than lubrication. **The anal glands of the Carnivora** are sebaceous glands, concentrated in number and developed in size. Associated with them are groups of saccular glands developed from the basal layer of the stratified epithelium. These glands are not very well adapted for lubrication purposes, and the fact that they are more or less odorous would lead one to believe that they served some end in the life and habits of the animal by giving off a distinctive scent. This idea is supported by a provable fact when we encounter these same glands in two of the mammals, the muskrat and the skunk. Here can be recognized, in structure, the anal glands of the other mammals, enormously enlarged, and producing a secretion that in the one case may be considered attractive and in the other is very offensive.

In section, these glands present but slight differences to the eye from the common sebaceous glands of the hair in the other mammals. They are somewhat larger, with larger cells and clearer cytoplasm, and are placed on special primary invaginations of the integument on each side of the anus. The saccular glands with simple secreting epithelium are placed in groups that surround the glands of the sebaceous type. They thus are collected into a single mass, the *scent glands*

(Fig. 364). The cells of the sebaceous glands produce an oil and produce it in the same way that the ordinary sebaceous glands produce it, as far as we can see. But the chemistry and the physiology of the

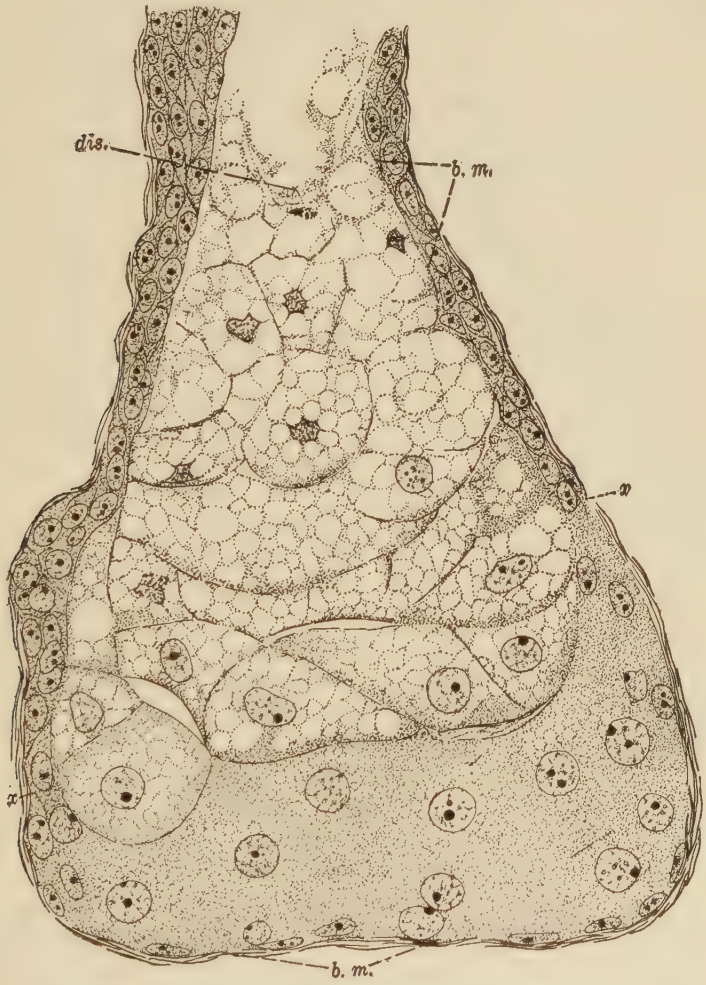

FIG. 364. — Axial section of a single acinus of the scent gland of a skunk, *Mephitis*. *b.m.*, basement membrane; *x*, boundary between secreting epithelium and stratified epithelium of upper gland and duct; *dis.*, distal surface of secreting epithelium where it becomes the secretion. × 650.

process must be different, for the oil produced is not a simple lubricant, but has volatile constituents that cause a most powerful odor. The secretion of the saccular glands is watery and either acts merely as a carrier for the odorous oil or is itself a constituent part of the scent-

producing discharge. Many other mammals produce fluids by very similar structures, which are attractive or repulsive. The musk ox, bat, etc., show these organs.

The Odoriferous Glands of the "Stink-pot" Turtle. — Among the reptiles are some that are offensive to the smell. One of such is the common stinking turtle of the eastern United States which, when captured or handled roughly, gives off a most disagreeable and offensive odor. To do this it discharges drops of an oily fluid from the ducts of two symmetrically placed glands or sets of glands that are placed just inside of the shell on each side of the body. These glands are developed ontogenetically by invaginations of the integument at the point where the duct opens, and they are evidently lined by a stratified epithelium derived from that on the outer integument of the animal (Fig. 365). This stratified epithelium is constantly proliferating as was also that in the rump gland in the bird, and as all stratified epithelia are. It is also secreting an oily fluid, but there are minor differences in the manner in which the two kinds of glands do this as well as in the product. In the turtle the stratified nature of the layer is obscured by the fact that there are only two distinct strata, an inner or proximal which represents the basal layer of a stratified epithelium, and an outer or distal stratum of many cells in thickness. The single basal layer is constantly dividing off cells which are pushed into the thick, distal layer of cells and which begin to accumulate the secretion as soon as they become independent of the basal layer.

FIG. 365. — Part of the wall of the stink gland of a turtle, *Terrapene odorata*. *b.c.*, layer of basal cells; *o.c.*, oil cells lying among the many serous cells; *m.s.*, matured serous cell with dead nuclei and ready to burst; *b.o.c.*, young oil cells on the base; *sec.*, secretion. × 650.

The secretion appears in a single vacuole on the distal cytoplasm of the cell, and this vacuole enlarges and swells the cell to very many times

its original diameter. The nucleus remains round and full and does not deteriorate in any visible degree. When fully developed, the secretion is in the form of closely packed granules, and the cells float free from the epithelium in the loose secretion that occupies the lumen of the gland. After this the granules dissolve into a fluid and the stroma of the cell may rupture, setting the secretion and the nucleus free, or it may remain intact for a long period, retaining the secretion and nucleus, which latter has by this time lost its internal structure while yet remaining round and full in outline. When the secretion is discharged, all the remaining cells are probably ruptured, setting the secretion free.

The above are the principal cells of this gland, but the secretion is contributed to by another set of cells in a very small degree. This latter kind is found at rare intervals in the basal layer as well as in the other layer of the epithelium of the gland wall. The secretion of these cells is a thick, heavy, yellow oil which is sometimes placed in a single large globule, but more often appears in smaller ones. A remarkable feature is that more of these cells with their golden-yellow contents are to be seen outside of the gland in the surrounding connective tissue than inside the basement membrane. The inside cells of this kind have more probably been basal layer cells that have developed the oil-secreting power than connective-tissue cells that have moved through the basement membrane, carrying their load of secretion with them.

These glands are present in most turtles, although not in all. They are either lubricating glands or scent glands in all the cases in which they occur, but are undoubtedly odorous in the animal in which we have studied them. Somewhat analogous glands are found in the integument of other reptiles, sometimes on very different parts of the body, as the glands on the jaw of the alligator and the glands on the thigh of the lizard. Some of these may not be glands for producing odor, but most of them probably are. The secretion, it must be noted in this case as well as in that of the skunk, is not discharged automatically, as in the lubricating glands of the mammals (sebaceous glands) but is retained, and discharged as needed.

In some of the Amphibia an integumental gland is used to produce an offensive fluid (said to be poisonous to some of its enemies). These glands are somewhat like the surrounding lubricating or mucous glands in the same animal's integument, and consist of single saccular glands lined by a simple columnar epithelium.

The so-called poison gland of *Bufo* will represent this class of tissue as found in both the urodela and anura. This gland is vastly larger than the mucous glands found in the same integument, although it is arranged on much the same plan. A portion of its wall is represented in Figure 366. The cells, of the single layer of epithelium which lines it, develop

a huge quantity of granular material which destroys the distal part of their cytoplasm and fills the entire lumen solidly with the secretion. This secretion is the offensive matter. The proximal parts of the cells remain undifferentiated, and form a lining of cytoplasm in which the flattened nuclei lie.

FIG. 366. — Part of the wall of a repugnatorial gland from the skin of a toad, *Bufo*. *sec.c.*, secreting cells; *sec.g.*, granular secretion; *mus.c.*, parts of two of the investing smooth muscle cells; *bl.l.*, connective-tissue layer containing the blood supply in capillaries; *conn.t.*, connective-tissue septum belonging to the corium and found between all the glands. × 1000.

Outside of the nuclear layer appears a single, close-set layer of smooth muscle cells, two of which are shown in oblique section in Figure 366. They serve to contract the gland when the secretion is to be discharged.

Covering the muscle layer is a light connective tissue which contains, between its thin outer and inner plates, a plexus of capillaries. Still outside of this is a septum of connective tissue which lies between all of these closely placed glands. A duct is present, in a more specialized form than in the neighboring mucous glands. It has a complicated set of muscles for controlling the discharge of the secretion. When the gland is discharged, its lining is destroyed and renewed by the growth of a bud from the side, as has been described by Esterly and others.

The secretion, when discharged, is a modified mucus, and is watery instead of oily, as in the preceding vertebrate forms. The organs have been considered as offensively odorous rather than poisonous, because the animal has no means of injecting the secretion into the blood of an enemy or victim.

Vastly different from any of the above are the various tissues found in invertebrate animals, and which are used to produce either attractive or repulsive odors. We naturally understand those found in the air-breathing animals best. There are undoubtedly many such organs to be found in the water-breathing animals that we do not know of and may never find.

It is among the insects that the greatest number and variety of odorous

glands are found, both offensive and agreeable to their enemies and friends. Some of these are external integumentary organs and others are internal glands derived from the integument. We shall treat of several of the prominent types here. The great variety and number of these organs is astonishing. There are literally thousands of insects that possess the structures in many positions on the body. In some they are temporary larval structures. In most cases, as far as is known, they are either integumentary invaginations or they are accessory anal or oral glands.

A fair type of **an integumentary gland producing an ill-smelling fluid is to be found in the earwigs or** Forficulidæ. The opening of these glands, which are found at points on the latero-dorsal surface of the posterior part of the abdomen, gives grounds for thinking that it is an integumentary invagination.

Dissection shows that the gland is shaped somewhat like a retort, or is "pear-shaped." The narrow, funnel-shaped neck opens on the exterior by a very tiny ringed opening in the chitin. The innermost layer is a thin chitinous membrane that is striated irregularly and which gives off plate-like and tube-like processes proximally into the underlying (outer) cellular layer. This layer is composed of cells which are directly continuous with the hypodermal cells of the outer integument, as also the inner chitinous layer is continuous with the cuticle of the body.

The next point is to see how the gland-cells are placed and how the secretion is freed. The cellular covering is composed of two kinds of cells (Fig. 367). The smaller are of no particular interest, and have small oval nuclei and transparent bodies whose boundaries are hard to see. The plate-like processes of chitin extend into the cytoplasm in places. The second sort of cells are very large and of great specialization and complexity. The nucleus is extremely eccentric and flattened somewhat. It is surrounded by a thin, clear space. The larger part of the cell body is occupied by a clear cytoplasm which contains and probably also secretes the ill-smelling fluid. The fluid is drained from the cell by one of the chitinous tube-shaped invaginations of the inner cuticle. This fine capillary lies with its end coiled up in the clear area of the gland-cell.

FIG. 367. — Two secreting cells from the stink gland of the earwig. *sec.r.*, secretion region of the cytoplasm; *t.*, parts of tubule by which the secretion is withdrawn from the cell. (After VOSSELER.)

There are not many of these large gland-cells. They are scattered singly or in groups through the membrane, and their combined secretions fill the flask-shaped gland sac. A muscle fiber holds the neck and regulates the discharge of the fluid. The secretion is a most pungent and offensive fluid. It is acrid and volatile.

The Hemiptera have in nearly all cases a pair of glands in the body which open as the earwig's did on the surface. The surface, however, is on the ventral side near the third pair of legs. These glands secrete a fluid which, while widely different in the many species, has a peculiar odor characteristic of all true bugs. In some cases it is intensely offensive, and in others it is actually fragrant or pleasing, having a "fruity" odor. The glands are of various shades of red and brown and yellow and represent a distinct type. We shall examine these **scent glands from a large species**, *Belostoma Americana*, in which they form two symmetrical, elongated sacs or tubes. The color is a very light yellow, and while the animal seldom uses them, when one is dissected and the tissue is cut, the odor is intense.

Sections show that this gland like that of the earwig is an invagination of the integument (Fig. 368). It is a far more involved gland, being an irregularly tubular gland, convoluted in some regions. Its walls are thick and lined on the outside by a very delicate connective tissue which holds the numerous tracheæ in contact with it.

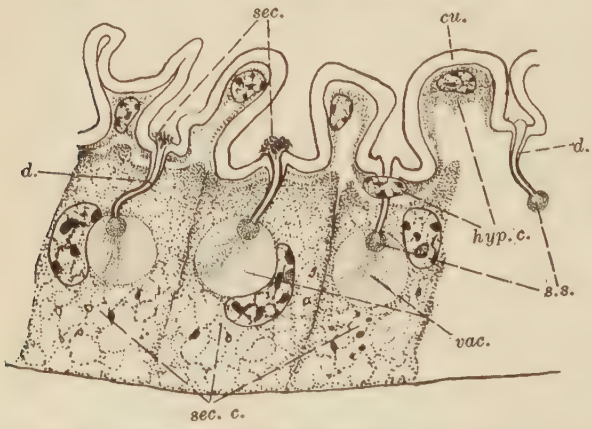

FIG. 368. — Bit of epithelium from the odorous gland of the Hemipter, *Belostoma*. *cu.*, cuticle; *hyp.c.*, hypodermal cells which secrete the cuticle; *sec.c.*, secreting cells, each with a large collecting vacuole, *vac.*; *s.s.*, secretion seive; *d.*, duct to carry secretion to the lumen; *sec.*, secretion emerging from duct. One seive and its duct is shown torn out and separated from the cell to which it was attached. × 1275.

The important layers of its walls are so involved that it is hard to describe any one without also describing the others. The innermost is a very much crumpled layer of chitin continuous with the exterior cuticle through the neck of the gland. At numerous points which are usually situated on the proximal flexures of the layer, are tiny openings into which the walls of the layer are produced downward as a fine, clearly marked tubule of even caliber and slightly curving

course. Besides these tubules, some plates of what appears to be chitin pass down between some of the cells.

The next two layers are cellular. That lying next to the cuticle is very thin and appears as almost a line except where its contained nuclei give it a greater breadth. It follows the sinuosities of the cuticle and its nuclei are placed most often in the upper curvatures. The tubules just spoken of penetrate this layer through or between its cells. It seems more probable that they pass through these cells.

Immediately beneath this layer is found the layer of secreting cells. They are thick and heavy, being roughly twelve to fifteen times the thickness of the chitinous layer and five to seven times its thickness in width. Some of the narrower ones are not secreting, and possibly may develop later into gland-cells. Those that are secretory show a very large, clear vacuole slightly above their middle height. This vacuole is marked off from the heavy, granular cytoplasm by a distinct membrane. It is evidently homologous with the clear cytoplasmic space described in the odoriferous cell in *Forficula*, but is a higher specialization. That in *Forficula* had no bounding membrane besides other differences to be noticed in the drawings and descriptions.

The cuticular tube, mentioned above as coming down through the hypodermal cells from the cuticle, enters into the top of a secretory cell and penetrates to the vacuole which it enters. Here it ends in a round knob and it also apparently ends blindly, for its lumen comes to a blind point in the center of the knob, and there is apparently no further opening.

Fine fibrils reach from all points of the vacuolar wall to this knob which is placed near its distal wall. They make a beautiful radial picture which is well seen in three places in Figure 368.

The cuticular tube obviously serves the purpose of conducting away the secretion. The fact that a simple tube can and does perform this function in the earwig adds to the mystery of why a round, heavy knob should be placed over the end of the same structure in *Belostoma*. That it does carry out the secretion in this animal seems more than indicated by the collected granular material heaped up in the cuticle flexure into which it opens.

A word must be said here regarding the origin of the secretory layer in this gland. If the thin, hypodermal layer directly under the cuticle represents a perfect layer and all of the ectodermal cells in the gland, then the secretory cells are mesodermal in origin and the penetration of their body by the cuticular tubule is a secondary relation. On the other hand, the presence of this tubule and the fact that the cells are secretory lead the writers to believe that the secretory cells are derived in the embryo from the hypodermal layer and have acquired their proximal

position by moving down out of this layer as do the acid cells from the epithelium of the gastric glands of the stomach of mammals.

The Myriapoda have odorous glands which produce hydrocyanic acid gas. *Julus* is the best example and the gland is exceedingly simple. It is again an integumental invagination, a simple, saccular form, the color of whose tissues gives it a dark, purple shade. Its duct possesses a most interesting closing device, as we must call it, because its elasticity causes it to remain closed until the muscle fiber opens it (Fig. 369). The wall of the duct is made of an inner chitinous layer and an outer cellular layer, the hypodermis. Near its exit, one side of this tube is evaginated into the lumen of the tube, and the lower end of this process is forced down into the external opening. This is its natural position,

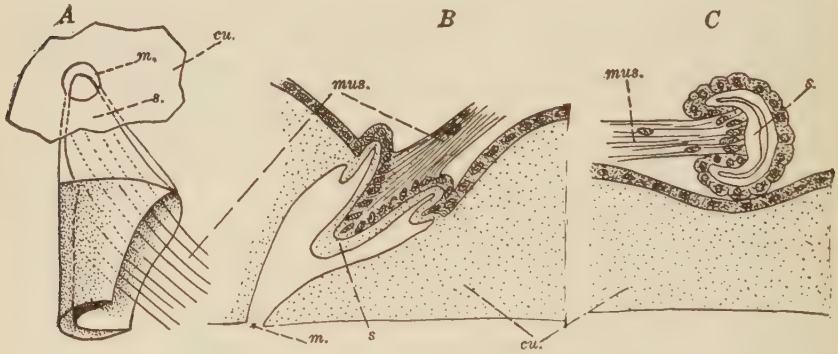

FIG. 369. — Three sketches to illustrate the apparatus by which the discharge of prussic acid from its repugnatorial glands is controlled by *Julus*. *A*, general side view with inside outlines indicated by dotted lines; stopper in place. *B*, slightly oblique section to show outer opening of duct and the stopper withdrawn. *C*, transverse section to show relations of muscle and stopper. Lettering for all figures: *m.*, mouth or outer opening of tube; *s.*, stopper; *mus.*, muscle by whose contraction the stopper is withdrawn; *cu.*, outer surface of cuticle, seen from the surface in *A* and from the side, in section, in *B* and *C*. (After ROSSI.)

and so the tube is closed and stoppered when at rest. A muscle fiber from some point of attachment on the ring segment enters the hollow lumen of this evagination, and when it pulls, the stopper is withdrawn, and the tube acquires a horseshoe-shaped lumen which permits the secretion to escape.

Beneath the cuticular lining of the sac lies the secreting epithelium. Unlike that of the two insect examples we have been studying, this is a simple, cuboidal epithelium. It secretes the poisonous and offensive hydrocyanic acid which one can smell when handling the creature.

Many insects give off an odor that is not only agreeable to other insects but to man as well. They are distinctly alluring glands. These glands are found in still more peculiar places in the anatomy of the creatures than even the offensive glands were. A favorite position in the

butterflies seems to be in various sacs on the body or in various positions on the wings. In these cases the hypodermal cells which formed the scales or hairs also secrete a fluid which is discharged through the scale in minute quantities, and vaporizes in the atmosphere.

While many invertebrates below the insects have peculiar odors we cannot discuss them as special odorous organs because enough is not known about them. This is especially true of animals living in the water, although it is satisfactorily proven that some forms of water animals have a keen sense of smell (selachian fishes).

Technic. — These tissues are usually fairly easy to cut, and paraffin section from Flemming and Zenker material show all that is to be seen with the exception of the innervation and, perhaps, blood distribution.

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TISSUES OF ADHESION AND SPINNING

These structures are found in a number of lower animals where they are fastened to a rock or other surface. They form a fairly homogeneous group of tissues and even the organs that these tissues compose afford a number of close homologies. The two principles involved in most of them are the development in a columnar epithelium of two features; a set of non-elastic fibrils to afford strength and points to attach, and the secretion of some tough, gummy substance to cause the cells to adhere. In larger forms, epithelial cells, which are usually invaginated into glands, are used to secrete an adhesive fluid that can be extruded as a thread which sticks and hardens. In other forms the principle of suction is brought into use and various suckers, pads, etc., are used to attach the creature, usually temporarily, to some surface. Mechanical grasping organs will not be considered here.

The Protozoa show many forms of this power which, unfortunately, cannot be properly studied on account of the weak development and

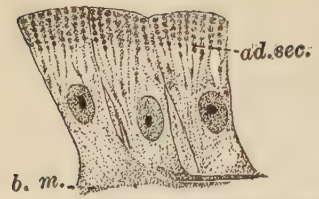

FIG. 370. — Three epidermal cells from the proximal surface of the "foot" of Hydra. *b.m.*, basement membrane; *ad.sec.*, granules of adhesive secretion on the distal parts of the threads. × 870.

specialization that is necessary in so small animals. The attachment is either temporary or permanent.

The Cœlenterates are rich in the number of forms that attach themselves by some part of their surface. *Hydra* will show an evident example of a typical form of adhesive structure. Sections of the epithelium on the "foot" show that this tissue has become thicker and stronger by the development of this power (Fig. 370). On closer examination one can see that the individual cells have well developed fibrils in their cytoplasm reaching from proximal to distal surface and better developed distally than proximally. This is a contrast with the few weak fibrils found in other epithelial cells on the body because these latter are far more irregular in direction and thus not so well fitted mechanically to bear a strain. The next point to be noticed is that in the outer fifth of the cytoplasmic body of the cell, a number of secretion granules appear lying against the fibrils. These are undoubtedly secretion granules of the adhesive material that is used to make the foot stick to the surface on which the animal rests.

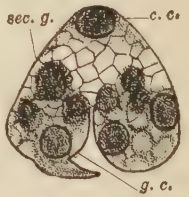

FIG. 371. — Very young stage of a group of grasping cells from *Beroe ovata*. *g.c.*, two young grasping cells; *sec.g.*, secretion granules; *c.c.*, single cap cell. (After SCHNEIDER.)

Other surfaces on the various Cnidaria have a similar structure, sometimes much specialized, but developed on the same principle. We shall go to the Ctenophora and examine a specimen of epithelium that is used, not to hold the body in any particular position, but to seize the prey upon which the animal feeds.

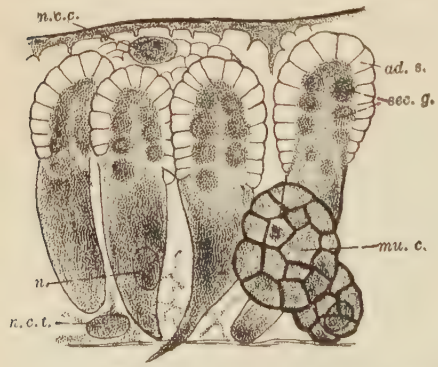

FIG. 372. — Half-developed group of grasping cells from *Beroe ovata*. *n.*, nucleus of grasping cell; *n.c.c.*, nucleus of cap cell; *n.c.t.*, nucleus of connective-tissue cell; *sec.g.*, secretion granules (poison?); *ad.s.*, adhesive substance; *mu.c.*, mucin cells. (After SCHNEIDER.)

The tentacular epithelium of *Beroe ovata* has been described by Schneider, and shows this seizing apparatus splendidly developed (Figs. 371, 372, and 373). The apparatus is not unicellular but consists of a number of groups of cells, each group consisting of from three to seven cells extending

from the mesoglœal line as fibers, almost to the surface. Straight and thread-like in their proximal course, they arise and become ribbon-like when halfway up. This ribbon-like part is wound in a spiral form around the nucleus.

Lying as a cap over the nucleus and the fibrillar portion is the glue-forming cytoplasm with the vacuoles of adhesive substance covering its outer surface. Just under these vacuoles lies a layer of large, round plastid-areas that represent some secretory activity. They have been suggested to be poison-forming areas of the cytoplasm, but they more probably represent preparatory stages in the formation of the glue substance. A very peculiar cap-like mass on the distal end of the oval nucleus is not as yet understood, and we can assign no function to it.

A few other cells are found in connection with these. Some epithelial cells are grouped about the bases of the adhesive cells and help support them. Outside of them is a layer of covering cells and during their growth a single "cap cell" covers the six or seven cells in the group and protects them until "ripe." A few slime cells are found near and among them.

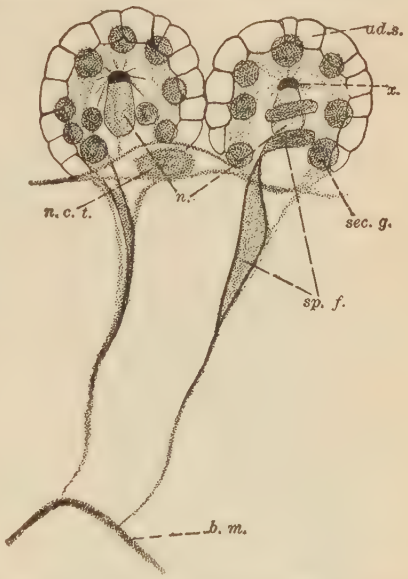

FIG. 373. — Matured grasping cells of *Beroe ovata*. *sp.f.*, spiral fiber which extends from the basement membrane (*b.m.*) and, ascending to the main cell body, winds in two turns about the nucleus; *x*, centrosome-like body on distal end of nucleus. Other letters indicate same as in the preceding figure. (After SCHNEIDER.)

Passing from the Cœlenterata to the Mollusca a somewhat **new type of apparatus for attachment** is found in which the second steps in specialization have taken place, the invagination of the glue-forming cells and the formation of this adhesive substance into strands used to attach the animal to its living place. This apparatus is known as a *byssus*.

Here it is the "foot" of the animal that develops the organ. This is not because the "foot" of the mollusk is homologous to that of the cœlenterate but because both of them are used to rest on and for attachment.

The common mussel, *Mytilus*, will provide a subject in which we may see a well-developed *byssus*. This should be dissected out with a scalpel, and also studied in a series of bulk-stained sections for a general idea of the apparatus. When it is desired to understand the histological

structure, it should be examined in a form in which the organ is not so highly developed, and where the cellular relations are more easily made out.

The byssus gland of the mollusk, *Ænigma ænigmatica*, as described by G. C. Bourne, is a very favorable object and Figure 374 shows part of a section through the byssus gland that shows the most important features. This structure is a deeply invaginated gland, extending in from the surface through a duct, which widens inside into a chamber of some size. This chamber is partly divided by a median septum into two lateral halves, and from the upper walls a series of thin lamellæ hang down, forming a laminated gland.

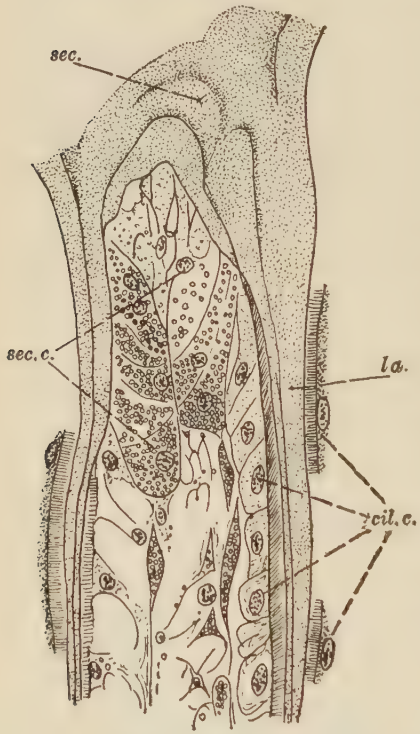

FIG. 374. — Vertical section through the edge and upper part of one of the lamellæ of the byssus gland of *Ænigma*. *la.*, lamina of secretion at *sec.*; *cil.c.*, ciliated cells lining the middle region of the sides of the lamellæ; *sec.c.*, upper or distal secreting cells. (After G. C. BOURNE.)

The secretion is produced by at least two sets of cells. The first part appears as a product of cells situated in the deep upper parts of the lamellar acini. This material is moved, as a thin sheet or *lamina* of the peculiar hyaline byssus substance, down towards the edges of the *lamellæ* or folds of tissue bearing the cells that produce it (Fig. 374, *sec.c.*).

The middle zone of these lamellæ bears flat cells whose surface is beset with short, stiff cilia. Some writers have denied that these cilia moved, and others have even disclaimed the idea that they were cilia. The writers believe them to be short, strong cilia which move the lamina of material downward. These cells secrete no material (Fig. 374, *cil.c.*).

When the laminæ reach the distal edges of the lamellæ they are added to by the epithelial cells of this edge which are enlarged and crowded full of "bissageneous" granules. Figure 374 shows this condition well. The various laminæ now fuse, in some cases remaining single, and form the well-known threads which attach the mussel to the rocks.

In other mollusks, as *Anomia*, the byssus substance is formed by

a shallow gland as a calcified shell-like structure which is fastened permanently to the rock.

The sucking disk of the fish-leech, *Pontobdella*, while used as a mechanical sucker, is also to some extent an adhesive instrument. The epithelium that covers the applied surface of this organ is stratified, and the small amount of adhesive material is more of a mucus than a glue.

FIG. 375. — Axial section through the grasping disk on the arm of a cephalopod mollusk; the squid, *Loligo Pealii*. *s.ep.*, simple epithelium becoming thickened toward rim; *sec.ep.*, secretory portion of simple epithelium, found on inner sides of cup to secrete the stiff cylinder (*cy.*) which is here distorted in the process of preparation; *p.ep.*, epithelium which acts as a portion or plunger to produce a vacuum; *mus.*, muscle which operates the piston; *ped.*, muscular pedicel (in outline) by which the organ is attached to the arm. × 600.

Several species of *Aphrodite*, another annelid, produce very large quantities of an adhesive material which is used to build the tubes in which the animal lives. This material is secreted by epithelial cells which are invaginated into glands. These glands correspond to the upper setigerous glands which, in other worms, are used to produce the solid setæ.

Another organ of attachment is to be found in the cephalopod mollusks. These are the "suckers" which are found on the arms of the various species of cuttles and octopus. They are outgrowths of the arm on its inner side, and each one consists of a cup-shaped organ of muscle and connective tissue, covered with an epithelium which is here simple, and attached to the arm by a strong, muscular stalk of pedicle (Fig. 375).

There are three regions of surface, each covered with a different kind of epithelium on the sucker-cup. The outside is covered with a simple form that is very thin on the proximal part of the outside, and which becomes thicker farther down on the outside, until it is thickest at the rim. On the broad edge it becomes very thick, and secretes a heavy, cuticular ring. This secretion is continued on the inner sides of the cup.

The bottom of the cup is covered with simple columnar cells that secrete mucus. This bottom is movable up and down in the cylindrical cavity of the cup which is kept rigid and open by the thick cuticle on its sides and edge. The muscle fibers which operate the epithelial "plunger" can be seen as parallel fibers passing from the outer surface of the bottom across to the inner surfaces of the plunger. A very narrow layer of muscle across the top, with its fibers lying in all diameters, serves by its contractions to compress laterally and thus to elongate the plunger.

When elongated, the whole lower surface of the cup is applied to any surface, and then the plunger muscle is contracted. This acts to produce a vacuum in the cavity, and to make the sucker adhere strongly. Weak circular muscle fibers are found in the upper part of the cup's sides. The figure is taken a little to one side of the stalk which, therefore, does not appear, with its core-like continuation, in the center of the suction muscle, but is indicated in outline.

A very remarkable **organ of adhesion is placed on the upper side of the head in the fish**, *Remora*. This sucker acts much as the squid's sucker did, but is more complex in structure. It consists of an oval, pad like area on the top of the head. The integumental edges of this pad are raised to form a rim, and somewhat similar folds run across its least diameter in parallel lines. Figure 376 shows part of a longitudinal section of the organ, showing one of the ridges. All folds, both the outer edge and the transverse folds are covered with the stratified epithelium characteristic of fish integument. This is the thickest on the edges of the pad and on the tops of the posteriorly slanting folds. It is exceedingly thin and weak in the hollows and on the under sides of the folds. It can thus be seen that the organ does not depend upon any adhesive secretion, and an examination of its mode of operation and muscular structure shows that it depends altogether upon suction.

The bed of the pad is a thick connective tissue in which lies a thin

plate of tough, rigid material, homologous in its structure and mode of origin to the fish scale. The folds each have for a central body a ridge of connective tissue, in the center of which are wide, spine-like projections that arise by a joint from the bed-plate and extend almost to the edge of the fold. Here they turn toward the upper surface of the fold, and frequently cut through the epithelium and project freely. The bed-plate consists of a number of jointed parts, and is backed by a muscular mass on the skull. Different branches of these muscles operate to raise the ridges and elevate the edge of the whole pad. The Remora

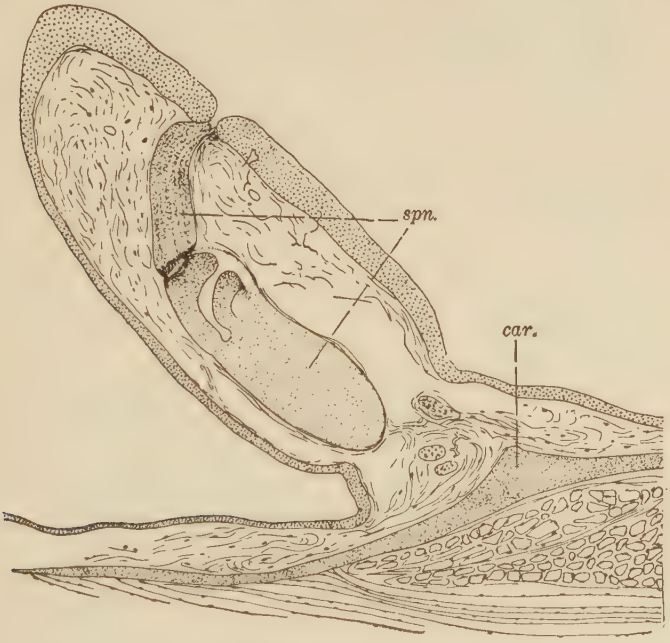

FIG. 376. — Longitudinal, vertical section through a small region of the grasping organ on the head of a teleost fish, *Remora*. One of the plates is shown and can be seen to be a modified fin ray. *spn.*, jointed spine; *car.*, cartilaginous base of organ. × 80.

applies its pad to the side of a shark, a swordfish, or a whale, and the muscles cause a rather weak suction to take place. The greatest suction occurs when the fish is drawn backward, as by the motion of its host, or otherwise. The projecting plates then tend to rise and thus create a vacuum under the pad which is made to adhere the firmer.

The feet of the insects show some notable examples of temporary adhesion by means of a gummy secretion. In the pine weevil, *Hylobius*, the entire sole of the foot is crowded by a great mass of the so-called "tenent hairs" which are single hypodermal cells whose bodies remain in the foot, and whose distal ends are produced into long, soft-walled pro-

jections which secrete a viscid fluid that causes them to adhere to whatever they touch (Fig. 377). These hairs are less abundantly developed in many other insects, sometimes to be used only during copulation or for other special purposes. In many larvæ the gland hairs are supplemented by various spikes and hooks, all formed from hypodermis cells and cuticle, as all other external arthropod tissues and organs are.

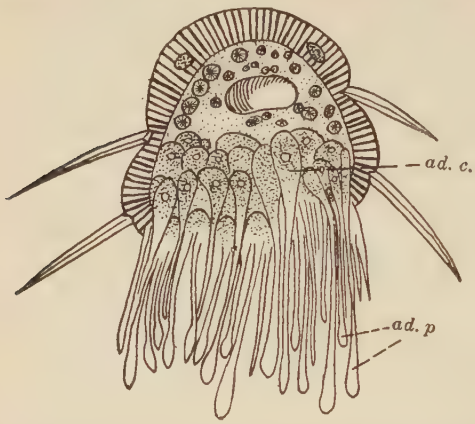

FIG. 377. — Adhesive structure on foot of a beetle, *Hylobius*. Transverse section of foot. *ad.c.*, adhesive cells whose processes (*ad.p.*) extend downward. (After SIMMERMACHER.)

One of the most highly specialized types of the organs of adhesion and spinning may be seen in the insects as a *spinning gland* of various larvæ and adult forms. These organs are invaginated portions of the mouth lining, and are well represented by **the spinning gland of the larva of a moth,** *Imperialis*. This gland has assumed the form of a narrow tube of some length and a portion of the wall of this tube is shown in Figure 378.

The cells are large and cuboidal. They form a single layer lying on a fairly thick basement membrane.

The cells are so large that they are supplied internally with tracheal branches. One of these appears in

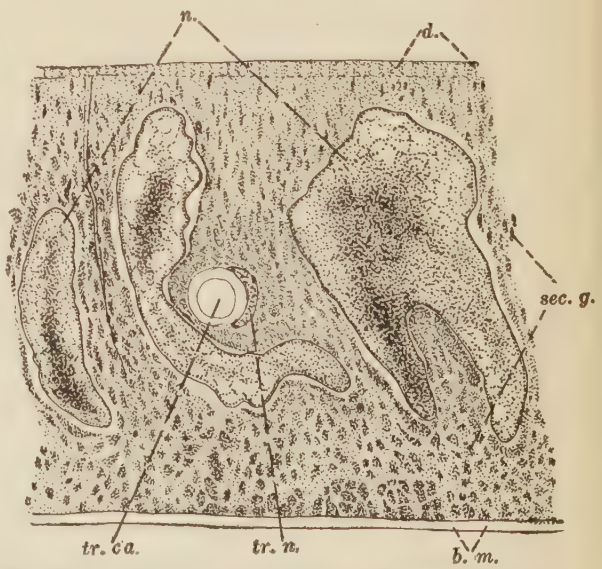

FIG. 378. — Portion of a longitudinal, vertical section through the epithelium of a silk gland in the larva of the moth, *Imperialis*. Three large, irregular nuclei (*n.*), representing two cells, are shown. *tr.ca.*, tracheal capillary with a single nucleus, *tr. n.*, in its wall (modified hypodermal nucleus); *d.*, distal edge of epithelium; *b.m.*, basement membrane; *sec.gr.*, secretion granules.

the section in transverse section, showing its surrounding covering of cells with a nucleus belonging to one of them. As may be seen in the figure the lines of demarcation of the cells are obscure or, in places, not perceivable. A marked peculiarity of all of them, in the insects, is the great branching of the single nucleus in each cell. The figure shows three sections of these branches, two of which belong to a single nucleus. The chromatin is finely granular, and a plasmosome does not appear in the section.

The cytoplasm is granular, and some of the granules are arranged in larger bodies much as they are in nerve-cells. These masses have an apparent axis which shows a tendency to lie parallel with the main axis of the cell. They are also larger in the proximal part of the cell and diminish in size towards the distal part. The distal surface of the cell, where it borders on the lumen, is coarsely but weakly striated. The nuclei of these cells should be examined in a freshly dissected gland from a cabbage worm (*Pieris*) to observe their peculiar shape.

The silk lies in the lumen as a thick fluid or semi-fluid mass. It is seen to have a peripheral layer of lighter material which may possibly be the fluid that is used to attach the main thread by, or may be a stage in the elaboration of the silk. The secreted material is used by forcing out a compressed end of this mass and passing it through the spinneret. It is used not only to build a protecting cocoon, but also to assist the animal to adhere and progress (in many ways).

Technic. — All these tissues cut fairly well with ordinary fixation and paraffin sectioning. It is always important to keep the secretion *in situ*, and to embed and cut so carefully that it does not fall out of the sections or shrink so as to distort the pictures produced. The combined paraffin and celloidin process is of use when the material is fragile; and when it is tough and refractory the celloidin process alone is of most use. In staining, it is well to use some stain that will differentiate the secretion material strongly. Most stains do this more or less, but some of the diffuse analine dyes, as eosine, are particularly favorable. With such stains the finer strands of the material can be traced to the cells which produced them.

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CHAPTER XXI

TISSUES OF REPRODUCTION, GENERAL OUTLINE

Among multicellular animals and plants the individual exists for a longer or a shorter period and then perishes. Certain cells, however, of these multicellular organisms may separate from the parent individual and, under proper conditions, give rise to new individuals. Through these cells, then, an unbroken chain of living cells is perpetuated. Cells so functioning are known as the *reproductive cells.*

Reproductive cells are of two kinds, *asexual* and *sexual.* The asexual reproductive cells are known as *spores.* It is characteristic of them that they may develop directly, without conjugation, into a new individual. Asexual reproductive cells and their organs are found in plants and certain very low forms of animals. We shall consider them only in the pollen cells of *Magnolia.*

The sexual reproductive cells are known as *gametes.* It is characteristic of gametes that without the union of *two* gametes or their equivalent, a new individual will not be developed. Certain apparent exceptions to this idea can be reconciled with it.

These gametes are different from any other cell or group of cells that may separate, bud, or divide from the body to form a new individual, in that they go through a peculiar process called maturation, which involves two cell divisions and a reduction of the number of the chromosomes by one half. In this case each cell does not develop into a new individual by itself, but joins with another reproductive cell, derived usually from another individual of the same species, and by a process analogous to a reversal of the process of amitotic cell division the two unite and become one cell, with the full number of chromosomes, which represents the beginning of a new individual of the species. This union is known as *conjugation.*

Every species of animal has its own kind of reproductive cell, but there also are two forms of this cell, a *male* and a *female* form. It is always a male and a female cell, usually from the same species, that thus unite to form the new individual, and besides any deeper or more significant difference between the two there is nearly always the comparatively superficial difference that the female cell, called the ovum, is the larger

and provides a store of food material for the first part of the future organism's life, while the male cell or spermatozoön is the smaller and has organs of motion which provide the means of moving through the usually short distance that separates the two when they are deposited for union, the female cell being passive.

When united, the ovum and the spermatozoön form a single cell, the *oösperm* or *zygote*, of at least equal value quantitatively and qualitatively to any cell in either of the parent organisms. This oösperm must be looked upon as the ideal cell, representative of its species, and it is by its subsequent divisions and the differentiation of most of its descendants, the somatic cells, that the new organism is formed.

Some of its descendants, however, do not differentiate but divide each time into cells exactly homologous to the oösperm (Fig. 379). These are the reproductive cells or germ cells of the organism and they form, as it can now be seen, an unbroken series from one generation to the next. They become smaller by dividing, but they divide nearly all their parts equally and are always the same. They become ready in the adult organism to grow in size, to mature, and to again part with their parent body, which they leave to die while they unite with another reproductive cell to form a new organism. Until the growth and maturation period arrives, however, the cells can be distinguished neither as male nor female, although their sex is probably already determined. They lie in more or less compact masses in various parts of the bodies of different animals, and form, together with connective tissue and other tissues, organs called the *gonads*.

The gonad may be a collection of tissues which develop *de novo* each breeding season to hold the reproductive cells or it may be a very permanent organ. It usually has associated with it a great complexity of accessory tissues and organs (genitalia) intended to facilitate the union of the spermatozoa with the ova and to further aid them in their reproductive career (brood pouches). A gonad containing male cells is known as a *testis* and one containing female cells as an *ovary*. Both male and female reproductive cells may occur in the same gonad or in different gonads in the same individual, in which case the species is known as a *monœcious* one. When the male and female cells are found in separate individuals it is known as a *diœcious* species. Both cases are common. The higher and more specialized kinds of animals are usually diœcious. The monœcious forms seldom unite an ovum and a spermatozoön from a single individual to form an offspring. An exchange of sperm between two individuals is the usual method.

The reproductive cells are the important or specific cells of a gonad, while there are several other kinds of cells that play secondary but necessary parts in its structure. Certain cells that feed the young

reproductive elements are called the *nurse cells* and they are usually former reproductive cells that at one stage or another of their development have given up their career as reproductive cells and devoted their energies to the nourishment of their more fortunate neighbors. The other gonad structures are the same tissues that are found in most organs, as connective, blood, muscle, and nerve tissues. They play the same part here as in the other organs.

Origin of the reproductive cells. — As stated before, the reproductive cells are the original and unaltered descendants of the dividing oösperm, the only ones of these descendants that have not been differ-

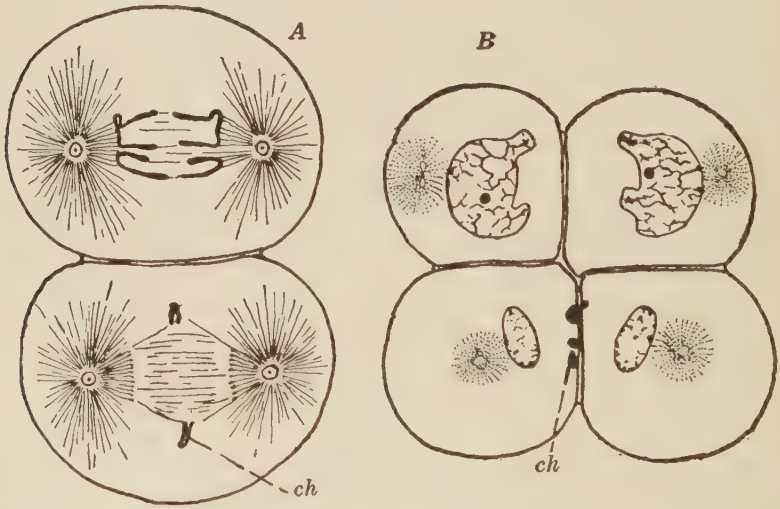

FIG. 379. — *A*, second cleavage division of the oösperm of *Ascaris*, showing the first differentiation by loss of chromatin (*ch.*) in the somatic cell. *B*, resulting four cells, showing the lost chromatin, *ch.*, and the smaller resulting nuclei in the daughter somatic cells. (From WILSON after BOVERI.)

entiated. This does not mean that changes of size and of arrangement of the cell-organs have not taken place, but it does mean that the cell has retained all its original powers and the necessary structures to exert these powers, which is not true of the somatic cells.

This loss of power by the somatic cell can even be demonstrated, in one case at least, to be a loss of some part of its chromatin and to occur in some species in the first cell division or shortly after. Such a solitary case of actual proof exists in the nematode worm, *Ascaris*, in which Boveri has clearly shown that, at the first division of the oösperm, one of the resulting cells is slightly differentiated from its sister cell by a potential loss of chromatin that occurs in the next division, while the other cell retains every feature of its parent, the oösperm (Fig. 379).

In this figure it can be seen that, while the long chromosomes of the upper cell in *A* divide, as is usual, by a complete splitting into equal halves, in *B* the chromosomes become separated by transverse breaks into many parts, and that when the division occurs, only the smaller middle parts are drawn apart to form the daughter nuclei; the longer end-portions being allowed to remain and become an inert part of the cytoplasm. In other words, one has become differentiated probably by the loss of some mechanism of use only to a reproductive cell. It has retained every feature but that one and possesses everything necessary to become any kind of a body cell or somatic cell. It has lost something that can never be replaced and it is destined to run a course of development and differentiation that will end in death for all its descendants. (Read Chapter IV for certain other developments of this idea.)

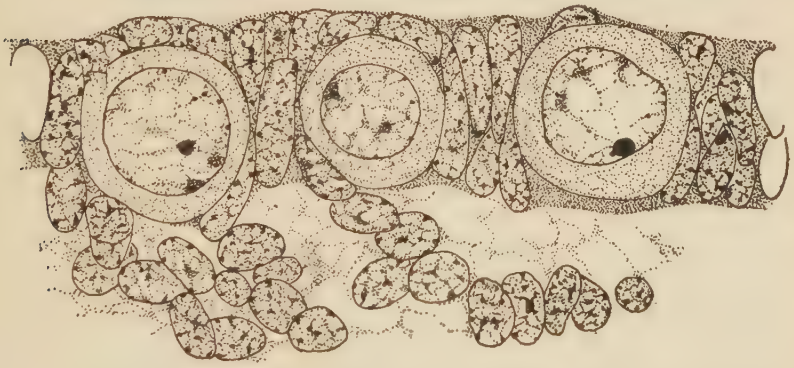

FIG. 380. — Bit of germinal ridge of a young *Acanthias* embryo (10 c.m.). Shows three large reproductive cells in the germinal epithelium. × 1230.

For a number of subsequent divisions the reproductive cell continues to give off one somatic cell at each division, while the first somatic cell can produce nothing but somatic cells. After this the reproductive cell disclaims all responsibility, so to speak, for the soma or body, and devotes its time to self-multiplication and the production of a few accessory cells that will be of use to it later in its life.

In most other animals this differentiation of somatic from reproductive cells does not take place, as far as we can see, at so early a period, although the latter can be traced back to a fairly early stage in many forms. Figure 380 shows a bit of reproductive tissue in a very young embryo of the dogfish, *Acanthias*, in which three germ cells are for the first time visible. In most other forms it is not until much later that the reproductive cells can be distinguished, sometimes not until the body is well outlined in structure. In some low forms most of the somatic cells appear never to give up their power of reproduction and are differentiated

sexually at any time during the adult life of the organism. This is true of most plants whose growing cells are also their potential reproductive cells, which, when the proper conditions occur, will mature into male and female gametes. In this case we find an organism which develops its gonads anew at each reproductive period of its life. The same facts are true of many sponges and cœlenterates and other low animals.

As a first step in the differentiation and higher organization of such plants and low animals we find that one or more of the somatic tissues lose the reproductive power. For instance, all of the tissues of a begonia plant, as stem and roots and even leaves, may be propagated to form a new plant which will produce reproductive cells. This power is lost to the leaves in a hickory tree, while the roots and stem retain it; in a hyacinth and some lilies and iris, the roots and leaves have both lost it and the power is confined to certain parts of the root-stock and the flower. It may even be lost to the flower in some highly cultivated plants that bear no fertile seeds.

The preceding paragraphs will themselves serve to harmonize to a large degree the view they express with a second view regarding the origin of the reproductive cells: that they are not cells of a separate line of descent, retaining powers that the somatic cells have lost, but that they may arise from the body tissues (usually described as mesodermal tissues) by processes of differentiation similar to those of any of the other tissue cells in the various tissues. These supposedly antagonistic cases merely show a later somatic differentiation: that the final differentiation may never occur, or it may be early, or it may be deferred until a comparatively late period.

As to the time at which sex is determined there appears to be more doubt and wider divergence of views as time passes and investigation along new and old lines proceeds. The idea that a high degree of nourishment during early embryonic life resulted in a majority of females and a low degree in more males has been shown to mostly mean a prior killing off of young females by starvation. Statistical methods and experiment along other lines have failed to throw light on the matter. Our chief hope of definite knowledge of more immediate causes seems to lie at present in certain cytological investigations on insects. This will be brought out in the following parts.

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GROWTH AND DEVELOPMENT OF THE MALE REPRODUCTIVE CELLS

The first stages of a male reproductive cell are represented, as are those of the female, by a rather larger and clearer mesodermal or ectodermal cell, which stands out from its fellows and must be identified by its position and the surrounding tissues rather than by its structure. Unlike the early ovum this cell has no great amount of food to store up in its body during the first part of its development. Certain of its fellows, however, are differentiated at an early period to act as nutritive cells to it during the last part of its development. As in the female, they are termed the nurse cells or, sometimes, the Sertoli cells.

The male reproductive cells are, at first, scattered through the future gonad or on its surface. As development advances they segregate into groups which are either rounded masses or elongate rod-like regions, as for instance the seminiferous tubules of mammals. We shall call these groups, irrespective of their shape, the *spermatic lobules*. As the sperm cells ripen the lobule either acquires a duct which conducts the semen away or else it ruptures and discharges its ripe contents into a body cavity or out into the surrounding water.

Most of these lobules are solid masses while the reproductive cells are young, and some of them continue so until the sperm is ripe and ready to be discharged, when the entire mass is allowed to flow into the sperm channels by the rupture of the lobular wall. Other lobules, which are solid at first, later acquire a lumen. It is only when the spermatozoa are beginning to mature that the lumen appears in the center. The presence of this lumen leaves the reproductive cells which line the lobule lying in a single or, more often, multiple row on the capsule, and we shall hereafter refer to them in this condition as the reproductive epithelium. This reproductive epithelium is further divided, in practically all testes of well-differentiated animals, into a series of cell groups which are of greater significance and more fundamental in character than the lobule. The lobule is more properly an anatomical feature, sometimes small, as in most Crustacea, and largest perhaps in some of the mammals, where it forms the long seminiferous tubules mentioned above. These more fundamental groups of male reproductive cells, which we shall call the *sperm columns*, are smaller groups based upon some nutritive relations to the nurse cells. They are also determined probably by the time that some particular group of spermatogonia initiates the maturation process. The sperm column is usually associated with a single nurse cell or Sertoli cell, although it may rarely have more than one such

nourishing cell. The lumen of a lobule will be considered as distal in direction and the capsule as proximal.

The male gonad differs histologically according to the season and the ways in which the sperm is matured. Some organisms mature but one lot of sperm in a lifetime, and others mature it from a very different and newly developed testis each year. In such a form we are apt to find a lack of the sperm column segregation and to find that each lobule matures all its sperm at once and in a mass. Such a lobule is not a permanent structure, but is destroyed immediately after the discharge of the spermatozoa.

In the other animals the sperm may be produced for long periods or even continuously, as in man. Here the lobule is usually a permanent structure and a residuum of living reproductive cells, as spermatogonia, is always to be found on the basement membrane. At certain periods, determined in man by "waves" of successive maturation periods which pass down the tubules, some of these spermatogonia begin to undergo maturation. As they begin to mature and develop they leave their basal position and move distally in successive layers, meanwhile going through the maturation stages, until when they arrive at the lumen they become functional male reproductive cells.

The nurse cells commonly remain on the basement membrane. This obliges the growing spermatids to move to them and remain in a proximal position until discharged. All the cells of a single sperm column commonly mature together. In the skate we find a long, seasonal, sperm-production period during which a series of new spermatic lobules are being continually formed from a germinative center. As these mature they move away from this center until, when ripe, they are ruptured and destroyed at the surface of the testis, setting the spermatozoa free into the seminal ducts. These lobules show a well-defined sperm column arrangement.

The development of a spermatogonium into four ripe spermatozoa is one of the most interesting of known cytological processes. The stages, divisions, etc., through which they go are exactly homologous to those to be later described for the female reproductive cells. We shall describe these stages simply and shortly at first.

Beginning on the basal layer as a *spermatogonium*, the cells await the breeding season, and having gone through the *contraction stage* or *synizesis* each one grows in size to become a *spermatocyte of the first order*. This spermatocyte now lies, as a rule, in the second layer of the reproductive epithelium and rapidly goes through with its first reduction division, which results in the production of two *spermatocytes of the second order*. The chromatin is arranged and divided as will be described in detail below and, sometimes without re-forming their nuclei, the second sper-

matocytes divide again, producing four *spermatids* which each possess one half the number of chromosomes that the spermatogonium did. These four spermatids all develop without further divisions into *spermatozoa*. We should now examine more closely as to what happens during the reduction divisions.

Maturation is a phenomenon common to both spermatogenesis and oögenesis and is an essentially similar process in either event. Two rapidly succeeding divisions, the *reduction divisions*, constitute the important phase of maturation. These divisions effect a reduction of the number of chromosomes by one half, and involve primarily a quantitative equal, frequently combined with a qualitatively dissimilar distribution of the fission products (chromosomes) among the resulting cells. The actual numerical reduction of the chromosomes has already occurred during synapsis when the chromosomes united into pairs, forming bivalent chromosomes, or several may even have combined to form plurivalent chromosomes.

It is now believed that the pairs of chromosomes in synapsis are composed of maternal and paternal elements and that their union represents the final stage in the fertilization process which resulted in the origin and development of the organism whose germ cells are now in synapsis. It is clearly known in several cases that the maternal and paternal chromosomes do not fuse at fertilization nor during the several succeeding segmentation divisions, and it is very probable that, in the germ cells at least, the chromosomes from the two parents do not fuse until synapsis.

Synapsis, as described in many insects and plants, usually takes place during the telophase of the final oögonial or spermatogonial division, though it has been observed to occur slightly earlier or later, sometimes even during the synizesis (contraction phase) of the chromatin at the beginning of the growth-period of the oöcyte or spermatocyte. According to the observations of various investigators in the various animal and plant groups, synapsis may be an end to end union of the elements of a pair of chromosomes or they may unite side by side. If they unite in the former way, we have a case of *telosynapsis;* if by the latter method, a case of *parasynapsis.*

It is known on good evidence, in some cases, that one of the maturation divisions separates entire chromosomes, and along the plane of their previous union in synapsis. It is only reasonable to suppose that the process is similar also in the more obscure cases. It is therefore essential for a correct interpretation of the maturation phenomena and the reduction divisions that we know how the chromosomes united in synapsis. This is known in but few cases. A maturation division that separates bivalent chromosomes into qualitatively dissimilar halves is known as a *reducing division;* a division that separates chromosomes

into qualitatively and quantitatively equal halves is called an *equation division.* If the division is of the ordinary mitotic type, it is known as *homeotypic;* if the prophase of the division is characterized by various ring- and cross-shaped chromosomes, the mitosis is said to be *heterotypic.* If the reducing division (frequently heterotypic) precedes the equation division, the case is known as *prereduction;* the reverse condition presents a case of *postreduction.* Both methods have been observed among animals and plants and with about equal frequency. Indeed, in some cases investigators disagree as to which is the method in the same species of animal. In very many recorded and well-authenticated cases, however, one of the maturation divisions is a reducing division and the other is an equation division.

In spermatogenesis, where the four cells resulting from the maturation divisions of a spermatogonium are all functional, the reducing division effects a qualitative inequality between the two pairs of fission products, as demonstrated by Wilson in certain Hemiptera; this dissimilarity may be in part a sex-determining factor, as McClung first suggested. The reducing division in oögenesis, where only one of the four cells resulting from the maturation process remains functional, effects the loss of chromatin to the ovum that may have represented sex determinants and various other ancestral hereditary characters. According to Weismann and his followers, the gist of the maturation phenomenon lies in the redistribution of the morphological representatives of hereditary characters, and offers the basis for variation and selection.

To take up a concrete example, let us consider **the maturation processes of the starfish**, *Asterias forbesii*, where the chromosomes are all characteristically dumb-bell-shaped throughout the maturation divisions. Here the somatic number of chromosomes is about 36. In synapsis, which probably occurs during synizesis, this number is reduced to 18, so that, with possibly one or two exceptions, the resulting compound chromosomes are bivalent. These chromosomes are typically bi-lobed. Since the details of synapsis in this particular case have not yet been observed, we have no clew as to what the maturation divisions mean where two longitudinal fissions are known to occur. But let us consider the various possibilities.

If the chromosomes united end to end in synapsis (telosynapsis) and condensed into a bi-lobed chromosome, so that one lobe is A (the parental chromosome) and the other is B (the maternal chromosome), then the first longitudinal division yields chromosomes that must be represented by AB, and the division was an equation division. The second longitudinal divisions would again result in chromosomes AB, and no true reduction would have taken place. Suppose the chromosomes to have fused side by side in synapsis (parasynapsis) and condensed

into a bi-lobed chromosome so that both lobes must be represented by *AB*. The first longitudinal division, if the separation really takes place along the line of original fusion, yields two chromosomes, *A* and *B*, and there has been a true reduction, and the case is one of pre-reduction. The second longitudinal division simply divides chromosomes *A* and *B* into similar chromosomes of half the original size, and the division is an equation division. In many cases one of the maturation divisions is transverse, but, however the divisions take place, the important fact as to whether the divisions are qualitative or quantitative merely depends upon the manner of union of the chromosomes in synapsis. These two important divisions have resulted in four *spermatids*.

The great temporary differentiation, which the male reproductive cell undergoes to adapt itself for motion, is now developed, and transforms it from a spermatid into the final form, the *spermatozoön* (Fig. 381).

The spermatozoön is a cell which has a variety of forms in different animals and these variations of structure can be best studied and understood when it is remembered that they have for a common object the transportation of the important nucleus of this cell to the ovum, its entrance into this ovum, and the final apposition of its chromosomes with those of the ovum.

There are two ways in which this transportation is accomplished: by the amœboid movements of an undifferentiated cytoplasm as in the spermatozoa of some Crustacea (Fig. 381, *H*); and by the development of one or more flagella or permanent cytoplasmic processes which propel the cell by swimming movements instead of by crawling, as must be the case in amœboid cells (Fig. 381, all forms except *H*). By far the larger number of spermatozoa are of the swimming kind, and most of these are propelled by a single strong flagellum. In the analogous male reproductive cells of plants there are oftener two flagella. We shall first concern ourselves with the structure of the more typical flagellate spermatozoön, as shown in the diagram to the right in Figure 381.

In this form the nucleus appears as a compressed oval body which is placed in an anterior cytoplasmic enlargement, the *head*. The nucleus is composed of the reduced number of individual chromosomes, of course, but they are indistinguishable at this time, forming a solid chromatic content of the nucleus. The head and its contained nucleus is not always oval, but may be much elongate, corkscrew-shaped (381, *D*), or formed like a horseshoe (381, *C*). The head terminates in a cap-like structure frequently sharp, occasionally blunt, called the *acrosome*.

The heaviest mass of the cytoplasm lies behind the head, and is known as the *middle-piece*. It is the cell body of the spermatozoön and contains the future centrosome, if this body is carried over structurally from generation to generation. The middle-piece is developed from the

nebenkern or *accessory nucleus*, a body found in the spermatid. The middle-piece contains a round body which is sometimes double and is called the *end-knob*. It is chromatic, and lies just behind the nucleus.

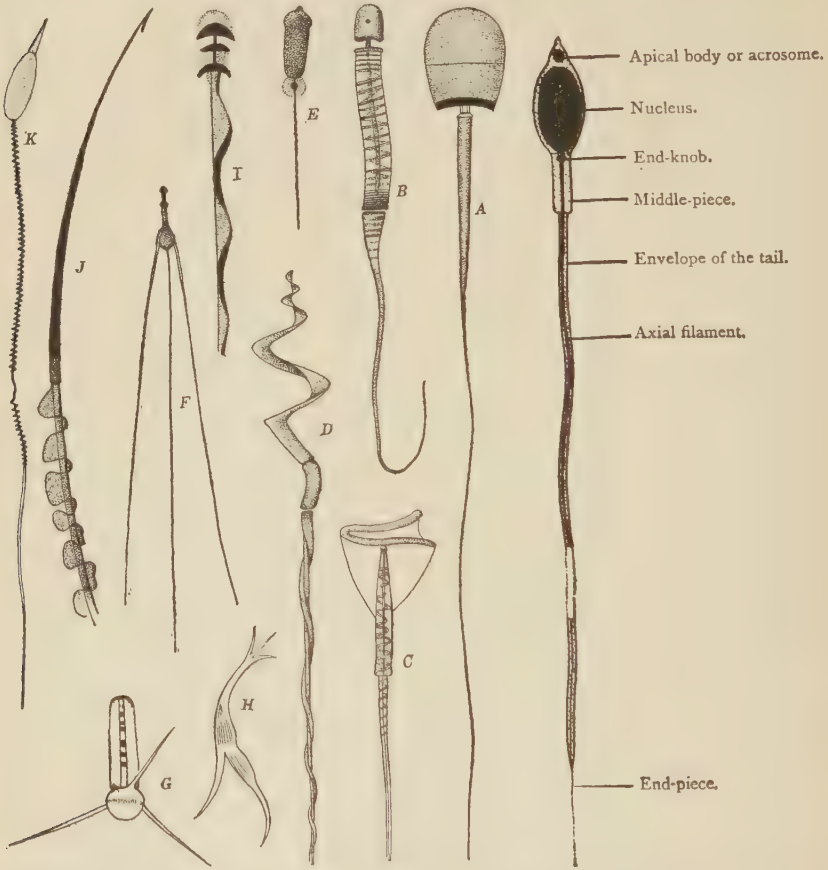

FIG. 381. — Labeled diagram of a typical flagellate spermatozoön, and figures of eleven actual spermatozoa to typify some of the principal forms. *A*, the badger, *Meles; B*, a bat, *Vespertugo; C*, opossum, *Didelphys; D*, a bird, *Muscicaps; E*, a sturgeon, *Acipenser; F*, a crab, *Porcellana; G*, the lobster, *Homarus; H*, a crustacean, *Polyphemus; I*, an insect, *Calathus; J*, a salamander, *Triton; K*, a snake, *Coluber*. (From WILSON after WILSON (*K* and *C*); BALLOWITZ (*A*, *B*, *D*, *E*, *I*, and *J*); ZACHARIAS (*H*); GROBBEN (*F*); and HERRICK (*G*).)

There is often a ring shaped structure associated with it. The middle-piece is sometimes much larger than the head (Fig. 381, *B*).

Running distally from the end knob, in our typical spermatozoön, is a thread-like structure known as the *axial filament*. This filament becomes continuous, at the end of the middle-piece, with the central portion of a single, long flagellum.

This flagellum, or *tail*, as it is known, consists of a strong axial filament which shows a fibrillar structure like that of smooth muscle. The cytoplasm covers it as a sheath, except for a part of its distal end, which has been called the *end-piece*.

Developed in or by the cytoplasmic sheath is a particular structure intended to give the tail a proper resistance to the fluid through which it swims. This is the *fin*, and may assume a number of peculiar forms. It is usually a filament or ribbon of considerable length, projecting as a flange from the cytoplasmic sheath around which it is spirally wound.

Many spermatozoa are of a widely different type of structure. The simplest is an amœboid form found in some Crustacea. Other Crustacea have very peculiar kinds, all of which seem to be constructed on a radial plan, with from three to twelve or more processes that cannot be called cilia or flagella on account of their structure. They are permanent cytoplasmic processes.

The changes through which the spermatid develops into the spermatozoön are well demonstrated in the following pages. We shall outline them in a few words at this point.

The spermatid is, at first, a very ordinary-looking cell, rather smaller in size than the average and with no suggestion about it of the spermatozoön form. Its first step in development is the appearance on one edge of a dot, the future *end-knob*, from which a tiny filament grows out distally. As the filament, which is the future *tail*, and the granule grow in size, they push in toward the nucleus, which the *end-knob* almost touches. A ring forms around the proximal part of the tail which now becomes the middle-piece, and the nucleus becomes compact and apparently loses its reticular chromatin. It moves into an eccentric position in the cytoplasm, and this region later becomes the head of the spermatozoön. The nucleus may assume a number of forms before finally becoming the head of the adult spermatozoön. The ring usually elongates and becomes spirally arranged about the tail. The axial filament has been shown to originate from a centrosome ray. Sometimes a secondary spermatocyte makes a weak attempt to form a tail in this way.

During this elaborate development of highly differentiated motor structures, the cell shows a varying ability in the power to nourish itself. Many spermatids apparently find no difficulty in securing food from the surrounding fluids, while others resort to the same methods that most developing ova do, and attach themselves to a nurse cell. In this case, however, one nurse cell becomes the feeder of many spermatids which, on account of their small size, need but little food. The connection is usually not established until the spermatid stage is somewhat advanced.

The relation is diffuse in some few animals (and most plants). This **diffuse connection by proximity can be well seen in the pollen sac of** *Magnolia*, where the outer members of a homogeneous cord of cells become the nurse cells and feed the inner cells of the same mass, which become the pollen cells (see Fig. 383). These pollen cells are formed in essentially the same way as are animal spermatozoa.

The diffuse relation between the source of nourishment and the nourished spermatids, somewhat as indicated in the above example, is the most general condition among lower, and especially the smaller, invertebrate animals.

Good concrete examples of **a more specialized connection are to be seen in the vertebrate animals.** Here the spermatids, soon after their development into spermatozoa is begun, form an attachment with a cell which lies near the basal layer of the reproductive epithelium.

They are drawn or move down between the other cells, and the head, of each one of a large group, becomes partly embedded in and firmly attached to the cytoplasm of this nurse cell which, in the mammals, is called a *Sertoli cell*. Here they remain until maturity, when they are released and set free to pass out of the reproductive lobule into the organs used for their distribution. This will be described in connection with the spermatogenesis of the skate.

FIG. 382. – Two sperm nurse cells from the salamander *Desmognathus fusca*. Each nurse cell has a group of half-developed spermatozoa attached and is feeding them during their growth.

In the salamander, *Desmognathus fusca*, a somewhat different method is used. As in the mammal, the spermatids attach themselves to the nurse cell, but the very large nurse cell is set free toward the end of the sperm development and floats about in the lumen of the lobule, feeding the spermatids until they are ripe. It then degenerates and is lost, freeing the spermatozoa. Figure 382 shows this condition.

The first series of developing reproductive cells that we shall study will be in a plant, *Magnolia soulangeana*. These asexual reproductive cells only indirectly give rise to male reproductive cells.

Here it is most clearly to be seen that four cells result from the two reduction mitoses.

This can be demonstrated clearly and plainly because at the beginning of this period each mother cell becomes incased in a tough-walled sac or cell-wall, and remains in this same envelope until its four descendants become full-grown pollen grains. This feature serves to show that the process of reduction in plants and animals is much the same and is possibly an homologous process.

The origin of the pollen mother cells is a more or less late (in the life of the individual tree) differentiation, which occurs each year from the ever young cells of the plant's upper growing point. As member after member, like leaf, bract, petal, etc., is produced by these cells, a time comes when the season or stage of growth determines the differentiation of a group of members called the flower, and in this group are certain members called the stamens.

Four longitudinal, parallel regions called the *pollen sacs* are early marked out in such a stamen member, and the cells within them become the primitive, pollen-forming cells. These cells are all alike at this time (early fall in Princeton) and lie dormant all winter, perhaps pursuing a very slow growth on the least cold days. In February, with the warming weather, the pollen sacs begin to grow, and now it will be noticed that the central cells are increasing in size much faster than the two outer layers. This is well shown in Figure 383, *A*, where the centrally situated

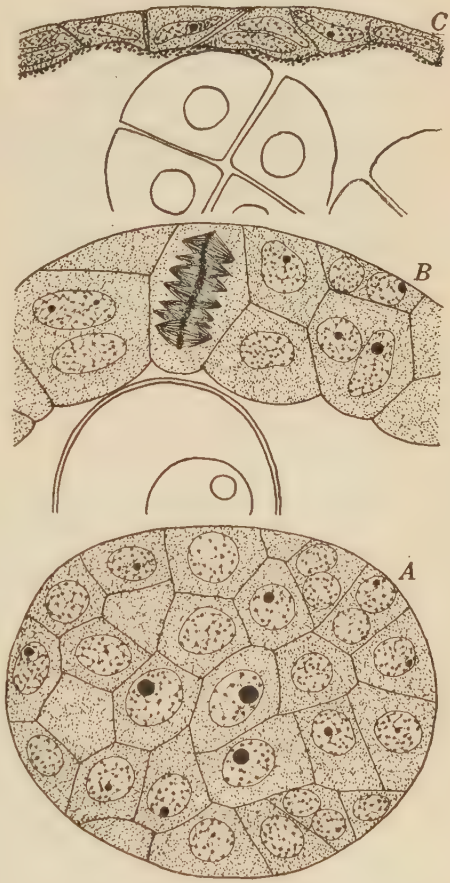

FIG. 383. — *A*, outer nurse cells in pollen sac of *Magnolia soulangeana* beginning to differentiate from inner reproductive cells. *B*, nurse cells in their vigor feeding pollen mother cells, one of which is shown in outline. One nurse cell in mitosis. *C*, nurse cells degenerating after pollen cells are formed. Two groups of pollen cells in outline. Low magnification.

cells in a transverse section of a pollen sac are larger and have proportionally far larger nuclei and nucleoli than the peripheral cells. These latter cells become differentiated as the nurse cells, forming at a later stage (Fig. 383, *B*) a double row of cells whose nuclei divide, by amitosis or sometimes by a peculiar many-centered mitosis seen in the figure, into two nuclei, without a subsequent division of the cytoplasmic body. Some of them already have two nuclei in Figure 383, *A*.

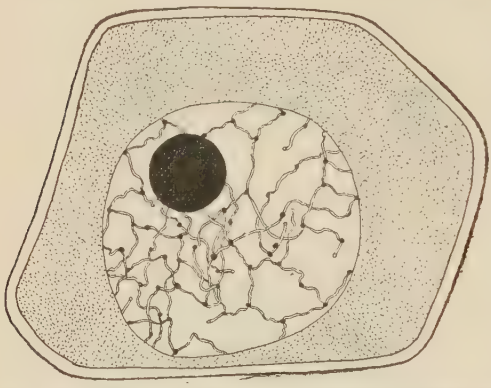

FIG. 384. — Pollen mother cell of *Magnolia* at period of completed growth and before any reduction processes have set in. × 1800.

Meanwhile the central pollen mother cells have grown to the proportional size indicated in Figure 383, *B*, where the outline of a full-sized pollen mother cell is seen in contact with the double layer of nurse cells, now at their fullest size and vigor. The double nuclei and the peculiar form of mitosis, which is rarely seen among them, are well shown in this figure. The ultimate fate of the nurse cells is shown in Figure 383, *C*, which represents the pollen sac at the time that each pollen mother cell has divided by its two reduction divisions into four young pollen cells. During this time the nurse cells have evidently been nourishing the reproductive cells, and now appear as a thin layer of shrunken cells, which are soon to further disintegrate and finally to disappear.

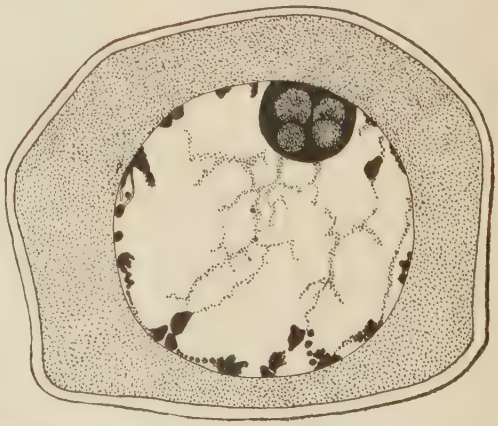

FIG. 385. — Pollen mother cell of *Magnolia* preparing for reduction divisions, nucleolus vacuolated. Chromosomes beginning to form. × 1800.

A pollen mother cell, such as is outlined in Figure 383, *B*, is better shown by Figure 384, which shows one at the maximum size of its growth and while it yet retains its primitive nuclear structure. The nucleolus is very large and very perfect in outline and shows no vacuoles. The skein which

appears is made up of a chromatic and an achromatic material, mostly the latter. It is smooth and wiry, with darker masses at the intersections of the strands of the skein.

Passing by several intermediate stages, Figure 385 shows a more developed cell in which it is to be seen that the skein is much broken up and its remains have been gathered against the nuclear membrane.

FIG. 386. — Pollen mother cell of *Magnolia* with nuclear membrane gone, nucleolus very small, and chromosomes forming. Achromatic fibrils are forming. *d.m.*, darker band of cytoplasmic material. × 1800.

These remains have also either acquired the power of staining, or they have had other material added to them to take the stain. This latter seems the more probable when we notice that during this time the large black-staining nucleolus has been going through a process of disintegration by the formation of vacuoles. While it appears larger on account of the vacuoles, it is undoubtedly smaller in bulk. This has not been demonstrated by any measurements, but is strongly indicated, if not proved, by the immediately impending disappearance of the nucleolus by this same method. The chromatic matter is thus gathered in irregular granules around the periphery and these granules increase in size as the nucleolus decreases in bulk. A small portion of achromatic material remains in a central position. The whole nucleus enlarges as this proceeds.

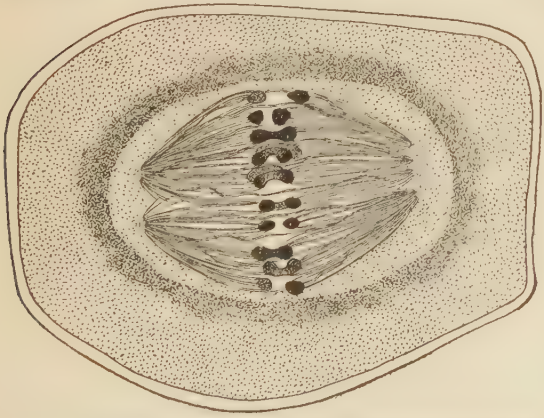

FIG. 387. — Metaphase of first reduction division in *Magnolia*. × 1800.

At the time at which the nucleolus is dissolved, or shortly before, a series of achromatic fibrils appear in loose formation around the edge of the nucleus whose wall becomes indistinct and disappears.

At the same time an equatorial band of darker material appears in the cytoplasm surrounding the nucleus (Fig. 386, *d.m.*); this band is cut at two points and the sections appear as two roughly crescentic lines of some width and tapering to blunt points. The chromatic material has mostly left the nucleus and been added to the chromatin particles which have now become larger, more uniform in size, and are evidently the future chromosomes of the first reduction divisions. They appear in the next stage represented (Fig. 387) in their regular size, shape, and arrangement, and the fibrils have been arranged into the familiar spindle which here is gathered distally into several points near together. The dark cytoplasm material is now increased to form a complete shell about the whole figure which is just beginning to divide its chromatin.

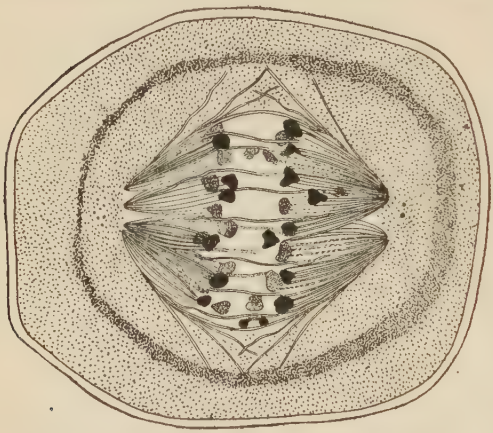

FIG. 388. — Anaphase of first reduction division in *Magnolia*. × 1800.

Figure 388 shows the division half done, and again shows the differentiation of mantle and spindle fibrils. The spindle fibrils seem to be fewer in this stage than in a later one (Fig. 389), where they are very numerous, and have already begun to show the equatorial plate that marks the position of the cell's final division. The chromosomes are still separate and are somewhat fused. The dark zone of cytoplasm is diffused and shows but a trace of its former presence. Radiating aster-like rays reach from the chromosome masses out into the cytoplasm.

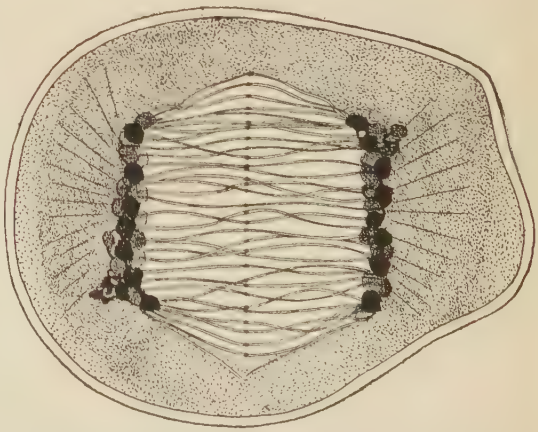

FIG. 389. — Late anaphase of first reduction division in *Magnolia*. × 1800.

In Figure 390 the two nuclei are re-formed and have acquired a very

much smaller nucleolus. A nuclear membrane is present and the chromatin is to be seen as scattered granules on the newly formed skein of achromatic material. The cytoplasm of the cell is constricted in the plane of future division.

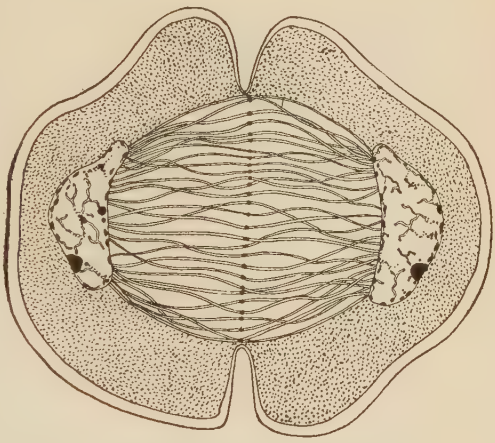

FIG. 390. — Telophase of the first reduction division in *Magnolia*. × 1800.

Before this cytoplasmic division has taken place, however, the second reduction division has started (Fig. 391). The two figures of this process occur at the same time and usually at right angles to one another. This is very convenient as a control for further observations. The darker cytoplasmic zone as seen in the first division, and which was lost in the short, intermediate, resting stage, is now re-formed around each of the two new figures.

The last figure (Fig. 392) shows the results of these two divisions, four small cells all lying in the same capsule or cell-wall which was formed by the original pollen mother cell division. The reduction divisions have thus resulted in *four* cells. The nuclei of these four cells but rarely appear in any one given section, owing to the position of the spindles by which they were formed being at right angles to each other. Thus the section from which Figure 392 was drawn was cut obliquely through the group, and two of the nuclei are only cut near their periphery and present a smaller section.

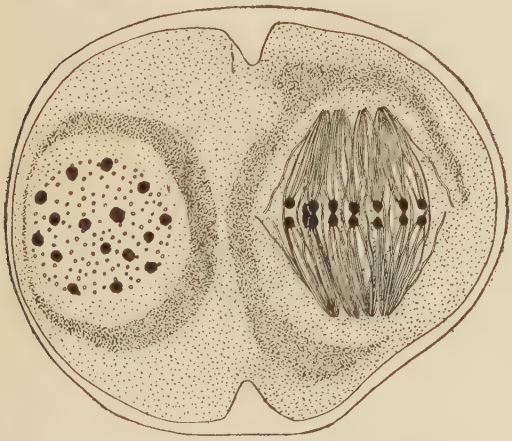

FIG. 391. — Beginning of the second reduction division in *Magnolia*. The two spindles have formed at right angles to each other, thus permitting two views of the figure in one section. × 1800.

The young cells now develop a cell-wall of their own of very peculiar pattern, with external spikes of knobs, and having broken out of the

original cell-wall which still incloses the four, they lie massed in the sac to await its ripening and rupture. The drying up of the scant remains of the former nurse cells sets them entirely free from any connection with the pollen sac.

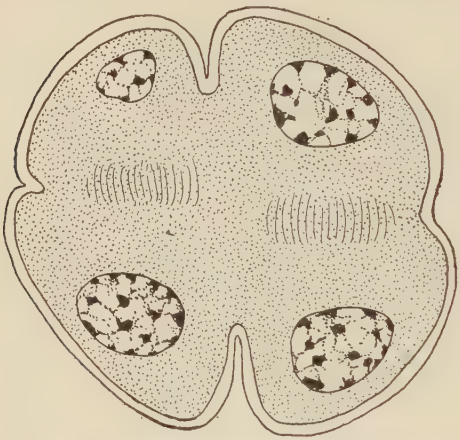

FIG. 392. — Youngest stage of the four pollen cells of *Magnolia*, which are all inclosed in the single cell-wall made by the pollen mother cell. × 1800.

The spermatogenesis of the skate, *Raja ocellata*, affords a very splendid object for demonstrating most of the details of sperm formation during which reduction takes place in the male gametes of animals. It illustrates at the same time several interesting histological methods of arrangement. The testis is a solid mass of tissue developed from the genital ridge, which is practically the same as in *Acanthias* (see Fig. 380). The large primitive reproductive cells of this ridge have multiplied and are situated, in the adult *Raja*, at a number of positions near the surface of the testis. They remain in these positions during the life of the animal and are constantly dividing so that they form small separate groups of germ cells which we shall call the *germinal centers*. In this center are a certain number of connective-tissue cells as well as the reproductive cells.

Each of these primitive reproductive cells is surrounded by several of the connective-tissue cells (Fig. 393). On the boundaries of the germinal center the reproductive cells may be seen dividing inside their enlarging connective-tissue cell coverings as in Figure 394, where each of the two groups of cells shown came from a single reproductive cell such as is shown in Figures 380 and 393. Thus each reproductive cell comes to form a spermatic lobule, which grows in size and matures its contents as it is pushed away from the germinal center by the formation of new lobules from this center. This whole mass of many lobules is surrounded by a capsule of connective tissue, forming one of the *lobes* of the testis. At the periphery of

FIG. 393. — Two primitive reproductive cells (pre-spermatogonia) in their resting stage in the adult testis of *Scyllium*. Each is surrounded by a few connective-tissue cells. (After MOORE.)

these lobes the sperm is constantly maturing during the breeding season and is then thrown off and collected for use through the *vas deferens*. When once a good section through a germinal center is found, it is comparatively easy to follow all the stages of sperm development by their comparative distances from this point as well as their actual structure.

FIG. 394. — Two very young spermatic lobules in the testis of *Raja ocellata*. The larger one shows a beginning of the lumen (*l.*) and a differentiation of nurse cells and spermatogonia. × 1000.

The single primitive reproductive cells seen in Figure 393 are probably a peculiar stage in themselves. We may call them the *pre-spermatogonia*. Each one is the originator of a single lobule, and all the reproductive and nurse cells in it. Its covering of several connective-tissue cells is a separate structure, and forms all subsequent capsule tissues. During the first divisions as pictured in Figure 394 it can be seen that the divisions of the reproductive cell have resulted in what can already be distinguished as larger, round, nucleated spermatogonia, and smaller cells with oval nuclei. These latter become the nurse cells.

In the skate and all elasmobranch fishes we have a case of early formation of the lumen in each lobule. This was already indicated in the larger lobule of Figure 394, and it is now very complete in the single lobule, one half of which is represented in Figure 395. The two kinds of cells are very irregularly arranged in the resulting germinal epithelium, but when growth has progressed to the stage seen in Figure 396, which represents a small portion of a section of one of the lobules, it can be seen that the reproductive cells all have a proximal position and the nurse cells have a distal position, forming a single row on the edge. A rather remarkable change begins to take place now, as is indicated in this figure by the withdrawal of two of the nurse cells proximally. This change consists in a migration of all of the nurse cells to form a single proximal layer on the capsule wall, leaving the many layered reproductive epithelium on the distal surface.

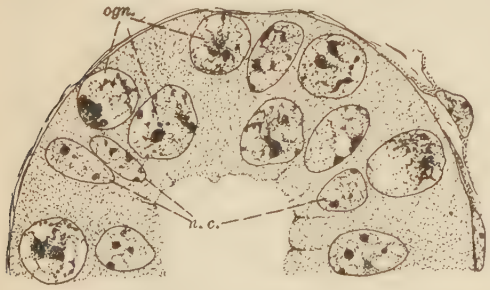

FIG. 395. — Section of one half of an older lobule than seen in Fig. 394. *n.c.*, nurse cells; *ogn.*, spermatogonia. × 1000.

The next stage (Fig. 397) shows spermatogonial divisions and at the same time the nurse cells can be seen in the midst of their migration.

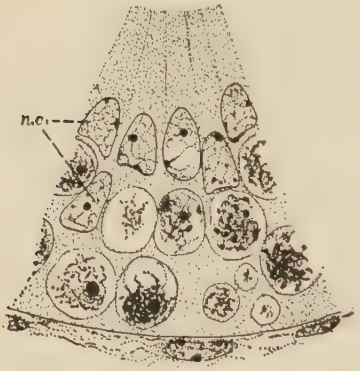

FIG. 396. — Part of a section of an older sperm lobule of *Raja ocellata* than that seen in Fig. 395. Nurse cells (*n.c.*) lie in a distal position. Spermatogonia numerous. × 1000.

FIG. 397. — Part of an older sperm lobule of *Raja ocellata* than seen in Fig. 396. Nurse cells (*n.c.*) migrating to a proximal position. Spermatogonia dividing. × 1000.

In Figure 398 this migration is finished and the reproductive cells (spermatogonia) have just finished their multiplication divisions. At this time they are from six to eight rows deep and must number in the neighborhood of 8000 spermatogonia for each lobule.

FIG. 398. — Part of an older sperm lobule of *Raja ocellata* than seen in Fig. 397. Nurse cells now all rest on proximal surface. Spermatogonia in synizesis or contraction stage. × 100.

The spermatogonia now go through the synizesis stage, or contraction stage, which is represented in Figure 398. After this they expand the chromatin thread and appear as the cells on the left of Figure 399. They are now known as the primary spermatocytes. In these it can be seen that the chromatin shows ring-like structures at places. These are the forming tetrads spoken of in the general discussion, and they soon become closely assembled in a dense, equatorial plate, which then divides by a pulling of the ring-shaped chromosomes into two halves. This may be seen in the right-hand portion of Figure 399.

The cells formed by this division are the secondary spermatocytes; they are shown in Figure 400. They are sometimes hard to distinguish from the early spermatids. It can be seen here that, for almost the first time, the germinal epithelium is being divided into the sperm columns. Two of these are to be seen in the figure, and at the base of each is to be seen a single, larger nurse cell, sometimes called in the male a *Sertoli cell*. The cell bodies cannot be separated by any line of demarcation, and the nuclei are large and lie flat on the basement membrane.

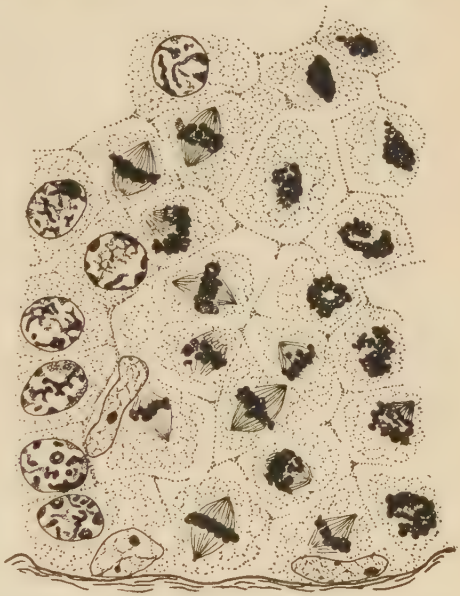

FIG. 399. — Tetrad formation and first reduction divisions in *Raja ocellata*. × 1000.

The reproductive cells soon divide again, first expanding their chromatin reticulum into a stage which is not figured. The divisions of secondary spermatocytes into spermatids are seen in Figure 401. They are far smaller than the previous division figures of the primary spermatocyte into two secondary spermatocytes. Moore describes the dividing chromosomes of this second division as separating globules, rather than rings which break in halves. The cell bodies are rounded and have become more separated from one another. No changes have occurred in the position or appearance of the nurse cells. The lower part of figure 401 shows some of these divisions in the latter stages. To the right are seen the results of these divisions, the youngest spermatids. The development of these into almost mature spermatozoa is shown in the next four figures.

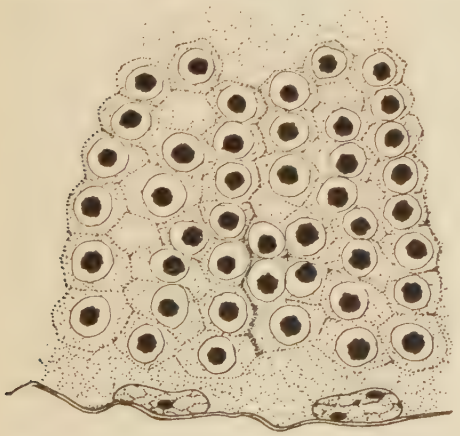

FIG. 400. — Second spermatocytes of *Raja ocellata*. Nurse cells on basement membrane. The reproductive cells begin to show a tendency toward the formation of sperm columns. × 1000.

FIG. 401. — Second reduction divisions by the secondary spermatocytes of *Raja oscellata*. × 1000.

Figure 402 shows two sperm columns, each extending distally from the nurse cell which belongs to it. It can here be noticed that each column is cup-shaped, and that its section forms an elongated loop with the bend placed against the nurse cell. The nucleus has moved to the proximal end of the cell in each spermatid, and a filament, the developing tail, passes distally from the nucleus out of the end of the cell.

Figure 403 shows a considerable advance on the last stage. The cells have all moved proximally, in the three sperm columns shown, and have gathered closely in the loop by the basal nurse cells. These latter have remained flat. Each spermatid has become much elongated and so has the nucleus, which is pointed distally and has a ring-like enlargement at its flat distal end. The cell outlines are poorly shown, but where cut transversely as shown in the upper part of the figure their outline is quite apparent with a dot, representing the tail, in the center of each.

The change from this last stage to that seen in Figure 404 is somewhat wide, but space forbids a closer comparison. In the latter stage the half-developed spermatozoa have greatly lengthened and have *all* moved down so that their sharp heads are buried in the nurse cells. This head, it will be seen, is the elon-

FIG. 402. — Two nurse cells and two sperm columns composed of young spermatids in *Raja ocellata*. The tails and end knobs are beginning to form. Cell walls are still complete. × 1000.

gated nucleus and its posterior end is much curled. The cell body extends, as a middle piece, back from the distal end of the head for about the same length that the head is. It invests the axial filament of the tail, and distally the tail emerges and, except for the fin which is not shown, is free for the rest of its course. The nurse-cell nuclei at this time have moved up from the flat position which they occupied before and lie alongside of the heads of the spermatids which are attached to them. They are rounder and fuller than before.

FIG. 403. —Spermatids of *Raja ocellata*. The nuclei are lengthening and the tails are forming. Above are a number of tails cut transversely and showing the outlines of the cells whose centers they occupy. These reproductive cells form two sperm columns, which are placed opposite to two large nurse cells on the basement membrane. × 1000.

The last stage to describe is shown in Figure 405. This shows how the spermatids have shortened in length, straightened the head, and been gathered into close bundles. Their points are still buried in the nurse cells, and the nuclei of these later cells are now large, full, and have well-developed chromatin structure, as well as a larger nucleolus than before. A rather remarkable vacuole appears at this time in the cytoplasm of the nurse cell and is filled with a dense mass that stains gray in iron hæmatoxylin. This mass rests against the distal end of the nurse-cell nucleus and is probably connected in some unknown way with the nutritive processes which are going on at this time. Other smaller granules appear in the proximal cytoplasm. It should be noticed in both this stage and in the last that the head of the spermatid is connected with the basement by a transparent strand of cytoplasm which passes through the body of the nurse cell. The spermatozoa are now nearly ripe, and it is in this condition that they separate from the nurse cell and pass out of the lobe with the rupture of the lobule at its surface. The nurse cells remain behind, showing more individuality at this time than at any other period of their lives. Three of them are shown by Figure 406 rising from the inner wall of a recently ruptured follicle.

The above description of the production of spermatozoa furnishes

a demonstration of the more ordinary features of this process in the animals. The more superficial features are discussed; but as in the majority of cases among animals the chromatin does not show all the changes it probably goes through, or allow us to explain the probable meaning of such changes as we can see. Dr. H. E. Jordan has very kindly permitted us to use the following account he has written, and the figures he has drawn of his studies on the spermatogenesis of *Aplopus Mayeri*, a giant "walking stick" of the Florida Keys, to demonstrate these features.

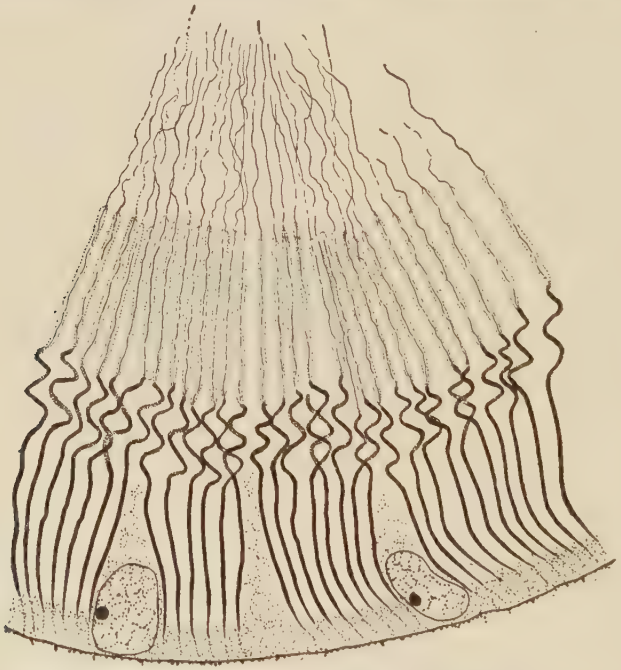

FIG. 404. — Half developed spermatozoa of *Raja ocellata*. Middle pieces are visible. The members of each sperm column have been drawn in or have moved in to bury their tips in the nurse cells. × 1000.

The accessory chromosome and its relation to the phenomenon of sex.[1] — The accessory chromosome (so named by McClung, '02) was first reported by Henking in a paper on the spermatogenesis of *Pyrrhocoris apterus* (Hemipter) published in 1890. Henking here noticed that in one of the spermatocyte divisions one chromosome did not divide, thus giving rise to two kinds of spermatozoa, one group with and the other without the odd element. McClung ('00–'02) studied a number of forms among the Locustidæ and Acrididæ, and reported uniform results in regard to the presence and behavior of the accessory chromo-

[1] Written by Dr. H. E. Jordan of the University of Virginia.

some. In the Orthoptera he was able to trace this chromosome back to the spermatogonial rest stage. McClung was the first to suggest a possible casual connection between the dimorphism of sex and the observed dimorphism of the spermatozoa. His conclusions were drawn from his observations on the maturation phenomena of the *Insecta*, and the fact that sex appears to be the only character that divides the individuals of a species into two approximately equal groups.

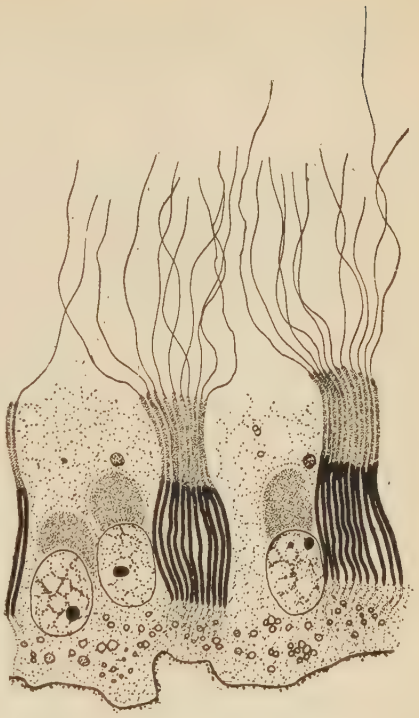

FIG. 405. — Fully developed spermatozoa of *Raja ocellata*. The heads and middle pieces have shortened and the sperm columns have become compacted into regular bundles. Peculiar bodies have developed on the distal end of each nurse cell nucleus. × 1000.

Recently there has been great activity in the study of the accessory chromosome and in a search for this element in the insects. It has been very thoroughly studied by Wilson ('05–'06) in several of the Hemiptera heteroptera (*Anasa tristis*, *Protenor belfragei*, *Alydus pilosulus*, *Harmostes reflexulus*, *Archimerus calcarator*, and *Banasa calva*). Here the accessory chromosome is associated with a pair of small chromosomes which behave differently from the ordinary chromosomes (they remain condensed in the growth stage) and are called by Wilson "microchromosomes." Miss Stevens ('06) has reported an accessory chromosome in *Aphrophora quadrangularis*, one of the Hemiptera homoptera (here also associated with microchromosomes) and in certain of the Coleoptera. An odd chromosome very similar in behavior to the accessory of orthoptera has been reported by Berry ('02) in *Epeira*, also by Blackman ('05) in *Scolopendra;* however,

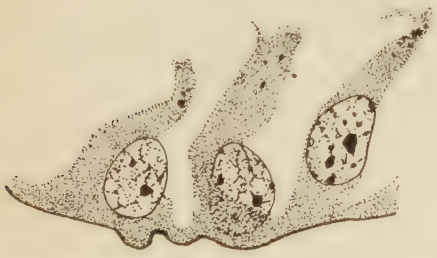

FIG. 406. — Three nurse cells on the basement membrane of a sperm lobule of *Raja ocellata*. The spermatozoa have been discharged from the lobule. × 1000.

the typical accessory chromosome (unassociated with micro- or idiochromosomes) is found only among the Orthoptera, where it has been described by Sutton ('00-'02) in *Brachystola magna;* by de Sinety ('01) in one of the *Acrididæ*, and several of the *Phasmidæ;* by Baumgartner ('04) in *Gryllus domesticus;* by Stevens ('05) in *Stenopelmatus* and *Blatella germanica;* and by Otte ('06) in *Locusta viridissima.* Moore and Robinson ('05) claim that in *Periplaneta Americana* the odd chromosome is merely a plasmosome, dissolving before each division and re-forming after it.

A different terminology has been employed by various writers to designate the accessory chromosome of McClung. Miss Stevens calls it the "odd chromosome"; Montgomery formerly used the term "chromatin nucleolus"; de Sinety designates it the "chromosome speciale"; and Wilson names it the "heterotropic chromosome," where it appears in the Hemipters.

Much theory and speculation has arisen in a regard to the accessory chromosome and its supposed connection with the inheritance and determination of sex. Castle ('03) has developed a theory of sex in which he applies Mendel's principle of segregation to sex phenomena. He shows that sex production may be explained as the result of a Mendelian segregation, transmission, and dominance of sexual characters. The theory has recently been more fully elaborated by Wilson ('06) and extended to apply to cases where either of the three forms of *heterochromosomes* (Montgomery) prevail: microchromosomes (small chromosomes); idiochromosomes (a pair of unequal chromosomes); or a heterotropic chromosome. The common character of heterochromosomes is their compact nature and deep staining reaction during the various stages of growth and maturation when the ordinary chromosomes pass into the nuclear reticulum. The theoretical discussion of the observations on the accessory chromosome and the far-reaching conclusions which may be drawn from the observed facts are better understood after a brief presentation of a concrete example. To this end *Aplopus Mayeri* (Phasmid), the giant walking-stick insect of Loggerhead Key, Florida, serves the purposes admirably. Here the accessory chromosome can be traced from its first origin in the secondary spermatogonial cells through the entire history of spermatogenesis into the nucleus of a half of the spermatids, where it finally disappears during the time that these undergo metamorphoses into spermatozoa. Except for the number of chromosomes, the facts, as they here obtain, agree in essential points with Wilson's latest report (after an extensive comparative study of smear, unfixed, and fixed and stained preparations) for *Anasa tristis.*

The primary spermatogonial cells (Fig. 407, 1) have a resting nucleus

with pale reticulum and an occasional karyosome. Nothing resembling an accessory chromosome or even a plasmosome can be detected. The cells divide mitotically (occasional amitotic divisions also occur)

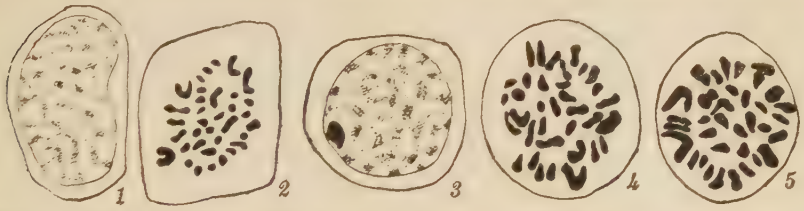

FIG. 407. — Male reproductive cells of *Aplopus Mayeri*. 1, resting primary spermatogonium; 2, chromosome count of dividing primary spermatogonium; 3, resting secondary spermatogonium showing accessory chromosome; 4, equatorial view of the 35 chromosomes of secondary spermatogonial division; 5, equatorial view of the 36 chromosomes of a dividing egg-follicle cell of female. × 1400. (Drawn by H. E. JORDAN.)

in the ordinary homeotypic fashion and the equatorial plate yields a chromosome count of 35 (Fig. 407, 2); none of these can be definitely identified as the future accessory. During the resting stage of the secondary spermatogonial cells (Fig. 407, 3), the accessory chromosome appears as a deep staining body amid the pale reticulum, situated close to the nuclear wall. Sometimes there appears a suggestion of a bipartite structure and occasionally even of a compact skein structure. The cells always divide by homeotypic mitosis, and the equatorial plates again contain 35 enlarged chromosomes (Fig. 407, 4). There are six or seven generations of secondary spermatogonia and only during the telophase of the final spermatogonial division (prophase of primary spermatocytes) does the accessory chromosome retain its compact form and deep staining capacity (Fig. 408, 6) among the ordinary, pale mossy chromosomes, and thus passes over, in original form, into the

FIG. 408. — *Aplopus Mayeri*. 6, telophase of final spermatogonial division; 7, resting stage of pre-synaptic period; 8, formation of chromatin lattice and lengthening of accessory chromosome; 9, spireme loops and accessory chromosome of pre-synaptic growth period; 10, opening of chromatin loops before synapsis; 11, synapsis of chromosomes. × 1400. (Drawn by H. E. JORDAN.)

resting or pre-synaptic stage of the growth period of the primary spermatocyte (Fig. 408, 7). Figure 407, 5, shows a metaphase group of a dividing follicle cell of the ovary with 36 chromosomes. The primary

spermatocyte now passes through a growth period during which it increases somewhat in size (Figs. 7–15).

The growth period presents various stages of intense activity and great protoplasmic alterations. After a brief resting stage (Fig. 408, 7) it enters upon a pre-synaptic phase, during which the nuclear reticulum becomes slightly chromatic and disposes itself into a lattice-work arrangement (Fig. 408, 8). At the same time the accessory chromosome lengthens out into a club-shaped mass extending through almost the entire diameter of the nucleus and becoming attached at its lesser end to the nuclear spireme. The spireme now breaks up into a number of segments (approximately 34) and these form loops at one pole of the nucleus (Fig. 408, 9). This represents *synizesis*. Subsequently these loops open up and one end becomes free (Fig. 10). The segments now unite in pairs by their free ends (Fig. 11) to form half the number of original loops. This stage is *synapsis* and, according to Montgomery and others, represents a pairing of homologous paternal and maternal chromosomes.

FIG. 409. — *Aplopus Mayeri*. Four stages in postsynapsis, showing postsynaptic reticulum and closing up of the elongate and ring-shaped accessory chromosome. × 1400. (Drawn by H. E. JORDAN.)

The large loops are the bivalent postsynaptic chromosomes. This end-to-end union is an example of telosynapsis. Meanwhile the accessory chromosome assumes a position to one side of and usually beneath the loops. Its longitudinal split opens up more or less completely during this stage.

During subsequent postsynaptic stages the chromosomes again arrange themselves into a reticulum (frequently giving indications of a longitudinal split) in the shape of a lattice-work. The accessory chromosome, meanwhile, closes up and shortens down into a compact, deep staining body closely applied to the nuclear wall (Fig. 409, 12–15). During the ensuing prophases (Fig. 410, 16–18) the spireme becomes split into a number of segments (17), each of which presently undergoes first a longitudinal, and secondarily a transverse fission, to form typical tetrads of various forms and sizes. While the ordinary chromosomes are at this stage, and still pale staining, the accessory chromosome is readily distinguishable as a body of deep staining capacity and sharp contour. It varies much in form, being bipartite, quadripartite, or U-shaped. A later prophase (Fig. 410, 19) shows all the chromosomes

deeply stained (many in typical tetrad form), among which the accessory is only occasionally recognizable where it has a large U-shaped

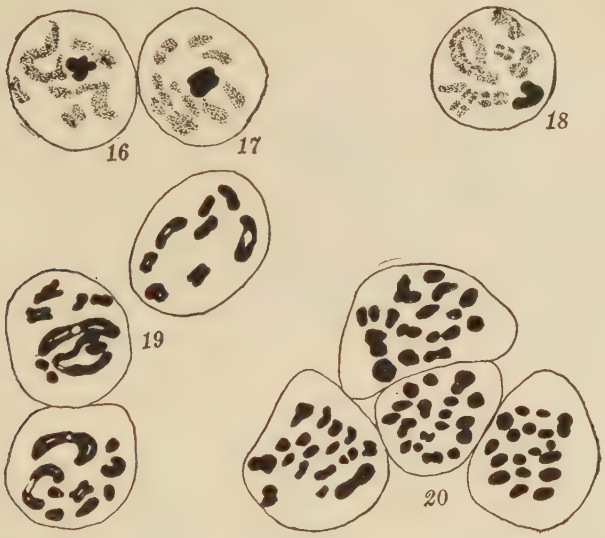

FIG. 410. — *Aplopus Mayeri*. 16, 17, and 18, three early prophases of the first reduction division, all chromosomes except the accessory are still light staining; 19, prophase in which the chromosomes are forming tetrads and the accessory is not recognizable because all the chromosomes stain dark; 20, equatorial plates of four primary spermatocytes about to perform first reduction division. Each shows 18 chromosomes, one of which is the accessory. × 1400. (Drawn by H. E. JORDAN.)

form. Figure 20 shows equatorial plates of four contiguous primary spermatocyte cells during the metaphase of the first maturation mitosis. Each of the cells has 18 chromosomes, one of which is the acces-

FIG. 411. — *Aplopus Mayeri*. Four stages in the first reduction division, showing how, in the operation, the accessory chromosome passes undivided to one of the daughter cells or sperm. *ac.*, accessory chromosome. × 1400. (Drawn by H. E. JORDAN.)

sory, not recognizable, however, among the ordinary chromosomes. It is one of the large eccentric bodies; other plates show the unmis-

takable U-shaped accessory chromosome in this position. In Figure 411, 21, are seen the ordinary chromosomes in the spindle at metaphase.

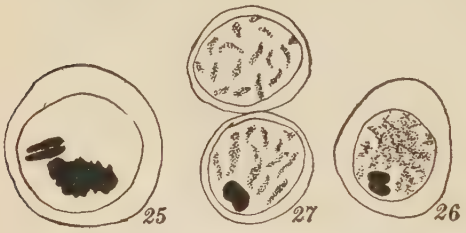

FIG. 412. — *Aplopus Mayeri.* 25 and 26, resting stages of second spermatocyte with accessory chromosome; 27, a pair of sister spermatocytes of second order, one of which has, and the other consequently has not, an accessory chromosome. × 1400. (Drawn by H. E. JORDAN.)

The first maturation division is undoubtedly transverse, separating whole chromosomes, and is therefore a true reduction division. The second maturation mitosis is the equation division (this being sometimes consummated precociously in the telophase of the first mitosis), both for the ordinary and accessory chromosomes. In the first reduction division the accessory usually assumes a position on the spindle in advance and to one side of the other chromosomes (Figs. 22–23). In the later telophase it almost invariably splits into two portions, the result of a separation of the two arms of the U at the bend (Fig. 24). Obviously, since the accessory chromosome passes undivided to one of the poles of the first maturation spindle, the resulting daughter cells (secondary spermatocytes) are of two classes, *i.e.* those with the accessory and those lacking it (Fig. 27). A brief resting stage ensues, during which the nuclear wall is reconstructed (Fig. 25) and the mass of chromosomes disentangles and diffuses into a pale reticulum.

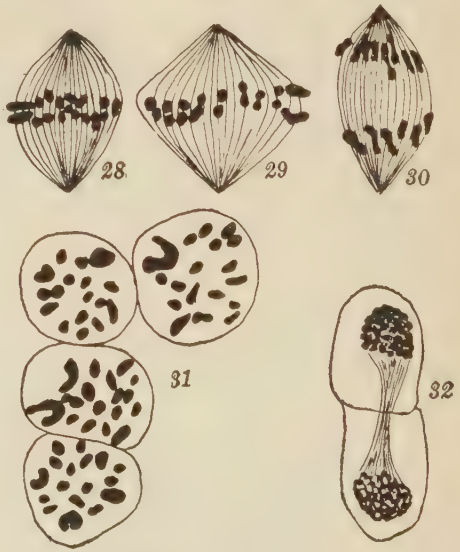

FIG. 413. — *Aplopus Mayeri.* 28, 29, 30, three stages in the second reduction division of the spermatocytes of the second order to form spermatids; 28 and 29 show equation divisions of the accessory chromosome; 31, equatorial plates of four secondary spermatocytes; two possess the accessory chromosome while the other two do not; 32, telophase of second reduction division; accessory chromosome not distinguishable among other dark staining chromosomes. × 1400. (Drawn by H. E. JORDAN.)

Meanwhile the accessory chromosome has remained separate from the ordinary chromosomes and has retained its characteristic form and position in the nucleus. Very soon the reticulum passes through the regular

prophase stages of fine, coarse, and segmented spireme (Fig. 412, 26–27). The stages of the regular homeotypic mitosis follow (Fig. 413, 28–30). A pair of chromosomes, larger than their fellows, lags behind in its entrance into the spindle and its passage to the poles in some of the cells. This pair, seen in Figures 28–29, are the products of an equation division of the accessory chromosome. Obviously again, equatorial plates of different spindles ought to show a chromosome count varying between 17 and 18, the latter count including the accessory chromosome. Figure 31 shows the metaphase groups of chromosomes of four contiguous, secondary spermatocytes. These are pairs of daughter cells of a pair of primary spermatocyte mother cells. The chromosome count alternates between 18 and 17 among the groups. The first and third are seen to contain a large U-shaped chromosome at the periphery of the complex. This is the accessory chromosome. Telophase stages of this mitosis (Figs. 32–33) do not reveal the accessory as a distinct body within the general chromatin mass; but as soon as the nuclear wall of the resulting spermatid is reconstructed, the accessory, of typical form and location, again presents itself in the pale staining reticulum (Fig. 414, 34). In Figure 35 is shown a spermatid in the first stages of metamorphosis to become a spermatozoön. It contains the accessory as a chromatic spherical eccentric body. A middle piece has grown out from a centrosome-like granule applied to the nuclear wall, and terminates in a long slender filament or tail about which later develops a cytoplasmic spiral fin. Later stages in the metamorphosis are shown in Figures 36 and 37, each figure representing a pair of spermatids with and without the accessory, respectively. Finally the accessory disappears in the head of the ripening spermatozoön.

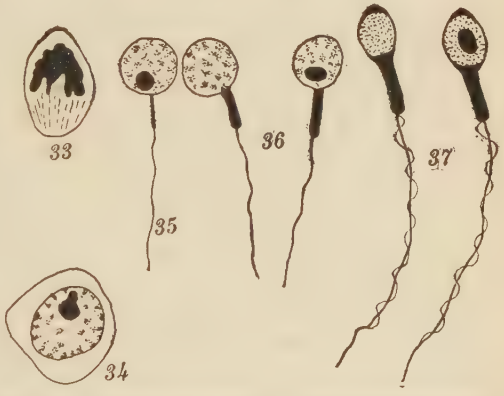

FIG. 414. — *Aplopus Mayeri*. 33, telophase of second reduction; no accessory chromosome; 34, spermatid, and 35, spermatozoön, with accessory chromosome; 36 and 37, a younger and an older pair of spermatozoa, one of each pair with and the other without an accessory chromosome. × 1400. (Drawn by H. E. JORDAN.)

The observed facts, then, that may be employed in forming a theory concerning the rôle of the accessory chromosome in the determination of sex as it has been developed by McClung, Castle, and most fully elaborated and most widely applied by Wilson are these: (1) an odd

number of chromosomes (35) in the spermatozoön; (2) an even number of chromosomes (36) in the follicle cells of the ovary (it has been proved in various forms of insects that the number of somatic chromosomes is the same as that of the presynaptic germ cells); (3) the appearance of a chromatin nucleolus in the spermatogonial cells which persists during the last mitosis preceding the spermatocyte phase and by its staining reaction and behavior during maturation proves itself a true chromosome (accessory chromosome); (4) the absence of a mate with which the accessory may pair during synapsis; (5) failure of the accessory to divide in the first maturation mitosis; (6) a resulting dimorphism of spermatozoa, consisting in the presence (18 chromosomes) and absence (17 chromosomes) of the accessory; (7) a reduction in the number of chromosomes, during the maturation of the egg, to half the somatic number (18 chromosomes).

From the above facts it follows that, if a mature egg possessing 18 chromosomes is fertilized by a spermatozoön with 18 chromosomes, an organism results which has 36 somatic chromosomes, and this is known by observation to be a female. Again if such an egg be fertilized by a spermatozoön with 17 chromosomes, an organism with 35 chromosomes results and this is known to be a male. The presence of an additional chromosome (the accessory) thus distinguishes the female from the male cell from the chromosome standpoint; hence the accessory chromosome appears to have some connection with the kind of sex that is to be produced. The final test of the theory that the accessory chromosome is a sex-determinant lies in direct experiment with that element in fertilization, the difficulties of which have thus far remained insuperable. Meanwhile, however far the theory may accord with fact, it remains a question, as Bateson has suggested, whether the accessory body may not be merely *associated* with the *cause* of sex; or, as Wilson suggests, the morphological expression of a hidden physiological cause. The most that may perhaps be claimed for the accessory, in relation to sex production, is that it represents sex characters, and this is further based on the assumption that the chromosomes really are the vehicles that carry the hereditary characters.

As alternative theories, Wilson suggests: (1) that the heterochromosomes may merely transmit sex characters, and that sex itself is determined by protoplasmic conditions external to the chromosomes; (2) that the accessory may be a sex determinant only by virtue of a difference in activity or amount of chromatin. Paulmier and Montgomery believe that the accessory is a degenerating chromosome and that its presence represents a stage in the evolution of a species from a higher to a lower chromosome number. They further believe, with Wilson, that it represents the persisting larger member of a pair of idiochromosomes,

the smaller member of which has disappeared. This suggestion is helpful in the further development of the theory of sex determination by the accessory chromosome. Again the elaboration and extension of this point has been made by Wilson. It was originally believed by McClung and Paulmier that, since the male germ cell carried the accessory body, eggs fertilized by spermatozoa containing this element should produce males. This conclusion was based upon an erroneous observation that the male somatic cells had more chromosomes than the female. Wilson has demonstrated in the case of *Anasa tristis* that exactly the reverse is true, and the same fact holds for *Aplopus* and other forms of insects.

Applying the same line of argument to *Aplopus*, and making similar assumptions to those suggested by Castle for an interpretation of sex along Mendelian lines: (1) two kinds of eggs (male and female) as also two kinds of spermatozoa which have been actually frequently observed; (2) selective fertilization or infertility of gametic unions of like sex chromosomes, *i.e.* an egg with a female determinant must be fertilized by a sperm with a male determinant, and *vice versa;* (3) the dominance of femaleness; also the further assumption suggested by Wilson; (4) that the accessory chromosome represents the larger member of a pair of idiochromosomes, the smaller member of the pair having been lost—it is then seen that, since a mature egg of 18 chromosomes fertilized by a spermatozoön lacking the accessory gives origin to a male, the male determinant must have been introduced by the egg and that the missing mate to the accessory was a female determinant. Furthermore, since when such an egg is fertilized by a spermatozoön possessing the accessory chromosomes a female arises, the egg must have contributed a female determinant to which the accessory or male determinant is recessive; it thus also appears that the accessory chromosome alternates between the two sexes in successive generations, *i.e.* the male contributes the accessory to the female in the production of the female, and the female in the ensuing generation contributes the accessory to the male in the pro-

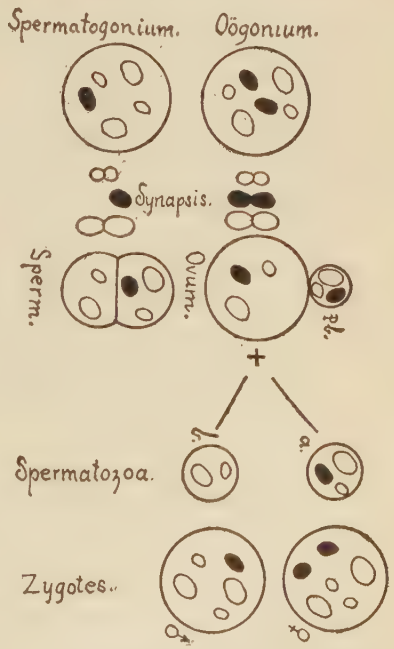

FIG. 415. — Diagram to illustrate the part played by the accessory chromosome in the determination of sex during maturation and conjugation. (After WILSON.)

duction of a male. This fact is admirably illustrated in the above diagram by Wilson (Fig. 415). Modifying Wilson's formula for sex production, to cover the case of *Aplopus*, the whole theory with facts and assumptions may be succinctly stated as follows: —

I. ♀ Egg (18 chromosomes) + (♂) sperm (18 chromosomes) = ♀ (♂) female (36 chromosomes).

II. ♂ Egg (18 chromosomes) + (o) sperm (17 chromosomes) = (♂) (o) male (35 chromosomes).

Technic. — Owing to the lack of yolk, and to the uniform protoplasmic texture of the specific male reproductive tissues, the technic has been easy, requiring only great care and exactness and a reasonable appreciation of the element of luck, represented by the unknown, and sometimes unmeasurable, factors which unite to make a successful or unsuccessful preparation. The various osmic and chromic fixatives, particularly Flemming's strong mixture and Hermann's fluid, have perhaps been most used, and with the greatest success. Corrosive sublimate and Zenker's fluid have been very successful in many cases. Smear preparations have been used in a few cases and ought, if used carefully, to be superior as a means of merely counting well separated and compact chromosomes. They cannot be trusted to show the relations of parts. The earlier stages of spermatogenesis have been observed during life by Wilson in the case of *Anasa*.

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GROWTH AND MATURATION OF THE FEMALE REPRODUCTIVE CELLS

The female reproductive cells are usually indistinguishable from the male, so far as any visible cytological differentiation is concerned, until near the time of yolk accumulation and maturation. They can usually be determined a long time before this by their position, or by the development of accessory sexual tissues in the organism that are not in direct contact with them.

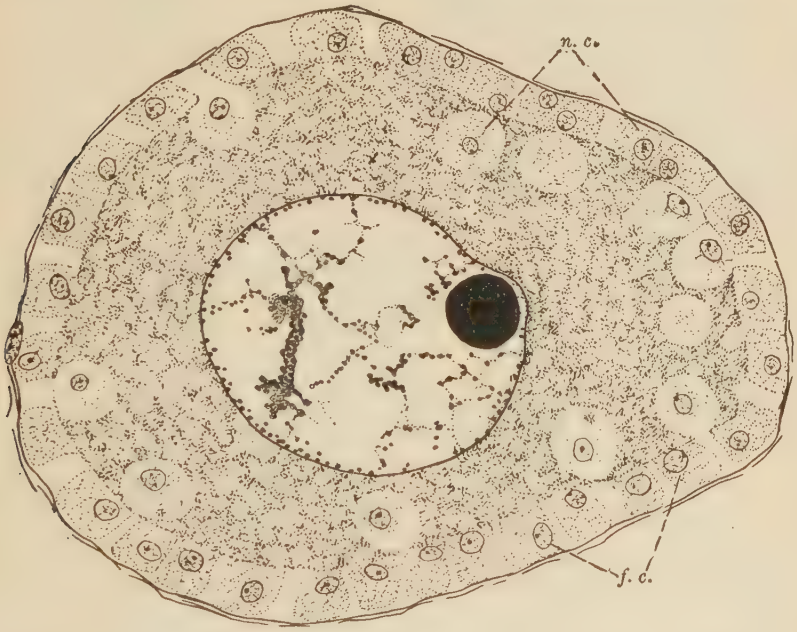

FIG. 416. — A half-grown ovum of *Molgula manhattensis*. *f.c.*, outer row of follicle cells; *n.c.*, nurse cells moving from follicle into cytoplasm of ovum. × 1200.

The first noticeable feature in the development of the young female reproductive cell is its relation to the cells which are going to aid it in securing and storing in its body the great amount of food material that will be needed later. It appears probable that, owing to its necessary occupation with its own internal preparations, or perhaps owing to the enormous quantity of food required, or possibly to the lack of food-preparing structures in its own make-up, that the ovum is always associated with such accessory *nurse cells*.

A primitive method of food acquisition is for the young egg cell to wander among the surrounding tissue cells and ingest them. This

appears to be the case in some hydroids. This method of ingesting food-laden cells is carried up into some higher forms as the tunicates, where the growing egg cell ingests whole rows of surrounding yolk cells whose nuclei persist for a while in its cytoplasm (Fig. 416). As a rule, where the cells are thus eaten bodily they are neighboring reproductive cells which are thus arrested in their career. This appropriation may even go on after the eggs are laid and development has commenced.

The more general method of yolk accumulation, however, is for the growing ovum to join itself in close bodily contact with some cell that,

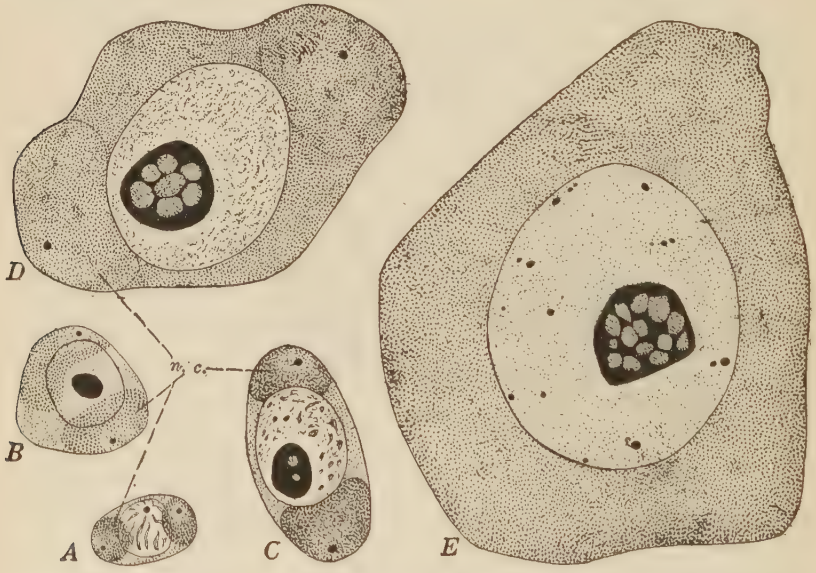

FIG. 417. — Five stages in the growth development of an oögonium of *Myzostoma*. The two nurse cells (*n.c.*) increase in size as the ovum grows and finally fuse with its cytoplasm. (After WHEELER.)

if related by descent, comes from a more remote common ancestor and is differentiated to act as a *follicle cell* or *nurse cell*. In this case the follicle cell or nurse cell takes food from the blood and, after elaborating it in some unknown manner, feeds it into the egg cell until there is a sufficient supply. It usually ends by giving up its own substance until but a dead remnant appears lying on the surface of the young ovum.

In the annelid *Ophryotrocha*, Korschelt has shown that **one single nurse cell** does all the work, attaching itself to the very small ovum and feeding it both with food secured from the surrounding body cavity fluid and with the contents of its own body until it appears as a mere excrescence on the body of the full-grown ovum, now many times the bulk of the two together when they started.

In *Myzostoma*, Wheeler has described the ovum as growing and securing its yolk supply through the agency of **two nurse cells**, one of which is attached to either end. Figure 417 shows five stages in this process and it can be seen that the nurse cells, unlike that of *Ophryotrocha*, grow in size as the ovum does and finally fuse with the cytoplasm of the full-grown egg. There appears to be a slight differentiation in the yolk which the two nurse cells produce, this differentiation resulting in a polarity of the ovum, which is retained through its further development.

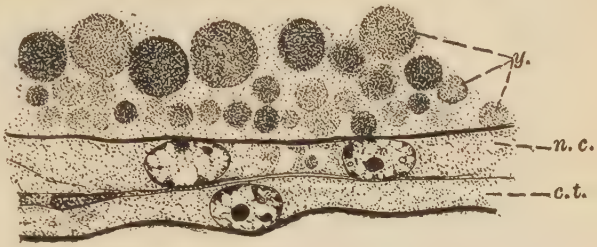

FIG. 418. — Two nurse cells of the single layer which feeds yolk into the growing ovum of the crayfish, *Cambarus*. *c.t.*, part of the connective-tissue follicle; *n.c.*, two nurse cells of the ovarian follicle; *y.*, yolk granules in the ovum. × 1300.

In by far the largest number of animals the yolk is prepared and loaded into the egg by **a single, epithelial-like layer of nurse cells.** This layer varies in thickness from the very thin layer found in most echinoderms through many grades of thickness and probably increased efficiency, to the long columnar cells that make up the nurse-cell layer of a siluroid fish.

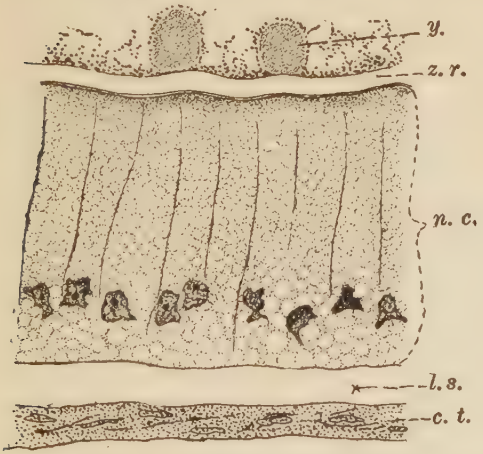

FIG. 419. — Part of the single layer of follicle cells which surround the growing ovum of the catfish, *Ameiurus nebulosus*. *c.t.*, connective-tissue follicle; *n.c.*, nurse cells of the ovarian follicle; *z.r.*, transparent zona radiata or cell wall of ovum; *y.*, part of ovum, showing two large yolk spheres; *l.s.*, lymph space (artificial or pathological?). × 870.

Omitting the case of an echinoderm because it is mentioned later, we shall demonstrate such a series beginning with **the yoke-forming nurse cells of a crustacean**, *Cambarus*, part of a section of whose half-grown ovarian egg is shown in Figure 418. The nurse cells form a flat, thin layer here of wide cells, with nuclei that are not much different from those of the surrounding tissues.

The food materials, from the blood supply which is constantly circulating through the surrounding space indicated by *l.s.*, must go through two layers of cells to get into the egg, a connective-

tissue layer lying outside of the follicle layer. It is probable that the entire blood soaks through spaces in the connective-tissue layer, and that only then do the nurse cells (follicle cells) select and elaborate the proper materials and pass them as yolk food into the egg.

The same thing is probably true of a very thick, single layer of cells shown by **the follicle layer of a catfish**, *Ameiurus nebulosus* (Fig. 419). While the ovum is very young, these cells are small and flat as in the crayfish. During the time of greatest yolk accumulation, the cells grow to the great height shown in the figure. The nuclei become smaller and angular, and a space appears between the follicle cell layer and the connective-tissue capsule. This space becomes filled with a heavy lymph of some staining power. The blood supply appears as a network of capillaries in the connective-tissue capsule instead of in sinuses outside of it as in the crayfish. Figure 420 shows part of a section of the ovarian egg of another teleost fish, the carp, and as this ovum was ripe and nearly ready to be shed, the follicle cells, which were almost as long as those of the catfish at an earlier stage, are now flat and shrunken and will soon die and disintegrate.

The cells of the single-layered follicle usually multiply by mitosis in the early part of their career. Later such cells, in some forms, divide by amitosis, which is a terminal process under the circumstances, as the layer is destroyed at about the time the egg is laid. (See Amitosis, Chapter V.)

We wish to call attention at this point to **the membrane which immediately surrounds the ovarian egg** in most animals and through which the food materials and other contents of the egg must be passed. In the catfish (Fig. 419) this membrane is comparatively thin, and one might think that the materials passed inside, in a fluid state, by osmosis. In the adult fish egg as pictured in the carp (Fig. 420), this membrane is very clearly perforated by a vast number of tiny, radial canals and the cytoplasmic processes of the nurse cells pass into these canals and thus carry the food matter in. The membrane is probably formed jointly by the follicle cells and the ovum, the latter being responsible for only the thinner inner layer. In some fish eggs the outer surface of the membrane is drawn out into long threads for attaching the eggs to seaweed and

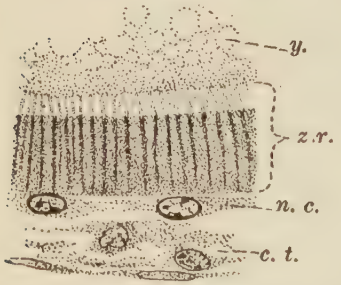

FIG. 420. — Edge of ovum of another teleost fish, *Esox Americanus*, to show the radial canals in the thick cell wall of the ovum. This cell wall is two-layered. The egg being ripe, the nurse cells are small and degenerating. *c.t.*, connective-tissue capsule; *n.c.*, degenerating nurse-cell layer; *y.*, yolk in ovum; *z.r.*, two-layered zona radiata. × 1300.

other foreign bodies. These are described by Eigenmann as being formed between the follicle cells.

A definite step in the organization of such a layer of nourishing cells is met with in the insects, where the egg may be said to be covered by **a single layer of nurse cells, one or more of which is enlarged** to perform the function of yolk storage. Where several cells out of the layer assume this rôle, they may continue to lie in a single layer or they may become arranged in a mass that is practically a stratified epithelium. A good subject to study is a "ground hornet," *Scolia dubia*. In this insect the ovarian tubules are terminated distally by chambers of primordial cells. A succession of single ova, each surrounded by a layer of follicle cells, arises from this terminal chamber and moves with the entire tubule toward the egg duct as an egg-follicle. Alternating with the egg-follicles are masses of nurse cells surrounded by a follicle epithelium to form a nurse cell follicle. Each nurse cell follicle is attached to its egg-follicle on its distal end (Fig. 421). The ovum sends a cytoplasmic process up into the fundus of the nurse-cell follicle. This latter follicle has increased in size by the growth of the nurse cells. As the nurse cells grow, their nuclei become irregular in contour and the chromatin breaks up into minute granules which are uniformly distributed throughout the nucleus. The cells which are first to so differentiate lie next the ovum (Fig. 422). This differentiation results in the secretory powers of the cells becoming active. A vacuole now appears in the egg cytoplasm, as though a streaming fluid had excavated the egg in this region (Fig. 422, *vac.*). The growth of ovum and nurse-cell follicle continues. Eventually a wave of cytoplasmic disintegration passes with some regularity through the nurse-cell follicle from the ovum distally. This is followed by the disintegration of the nuclei.

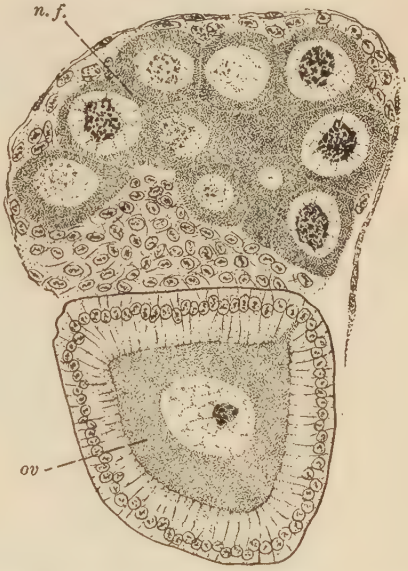

FIG. 421. — Young egg-follicle and attached nurse-cell follicle of the ground-hornet, *Scolia dubia*. *ov.*, ovum surrounded by a single layer of passive (?) follicle cells; *n. f.*, follicle composed of young active nurse cells which were derived from a part of the single layer. ×350.

The destruction of the contents of the nurse-cell follicle causes it to contract and finally disappear as an appendage of the egg-follicle. Fig-

ure 423 shows the destruction of the contents almost completed. In the earwig, *Forficula*, but one follicle cell becomes a nurse cell. In other insects, as *Vanessa*, several follicle cells are so modified, but remain an integral part of the follicle and are not separated from it as in *Scolia*.

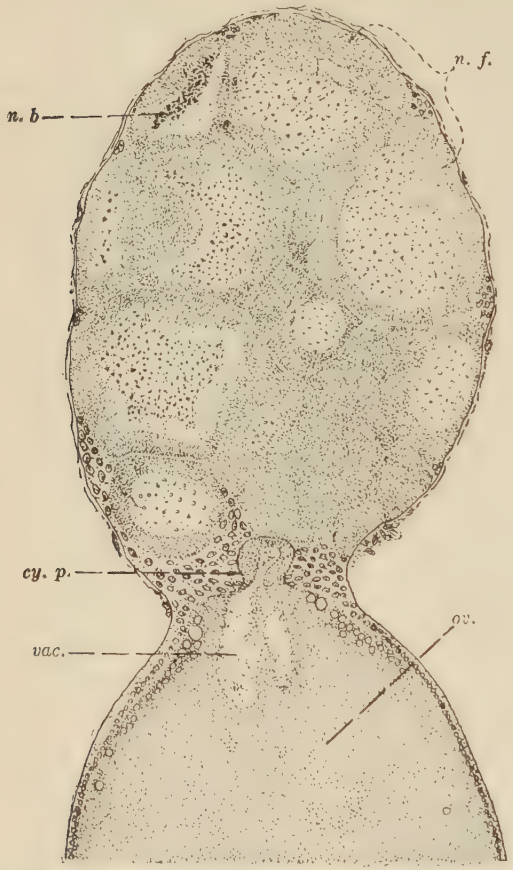

FIG. 422.— Older ovum of *Scolia dubia*. Lettering same as in Figure 421. At *n.b.* the chromatin has not yet become distributed as it is in the very actively secreting cells. *cy.p.*, cytoplasmic process of ovum through which the nourishing matter is drawn as a streaming vacuole (*vac.*). × 350.

Multiple layers of follicle cells are confined to the higher and most highly specialized animals and are instructive in that they show the necessity of bodily contact between the ovum and the yolk-supplying cells. Figure 424 shows the layer of yolk cells surrounding the growing ovum of a water snake, *Natrix sipedon*. When the ovum first begins to grow, this layer is single. The cells increase in size and form a stratified layer by amotitic proliferation. This layer is thickest just before the ovum has attained its full size, and when this size has been secured, the follicle cells degenerate into a layer of dead cells, which form a mucous covering to permit the ovum to slide out. The figure shows the most actively secreting stage. Study the middle layer and note that although the layer is stratified, yet every cell secreting has a strand of its cytoplasm drawn out into a process, the *yolk process*, which comes into contact with the ovum by passing through the egg membrane. This provides a necessary pathway for the yolk material, in solution or in fine granules, to be carried into the egg without being passed from cell to cell. Note also that the larger cells show a mass

of food material being elaborated in the cytoplasm distal from their nucleus.

The extreme outer layer is distinguished by its lack of food materials and is evidently not so actively engaged as the middle layer in which the largest cells lie. The innermost layer also contains smaller cells.

These cells multiply by mitosis during the earlier development of the ovum. A mere increase in the size of the individual cells appears to give the follicle sufficient capacity when the maximum growth is attained. In the mammals the multiple follicle layer continues to increase the number of its cells by mitosis up to the full maturity.

FIG. 423. — Part of nearly matured ovum of *Scolia dubia* with the attendant yolk follicle used up and degenerating. All communication between ovum (*ov.*) and yolk follicle (*n. f.*) is cut off. × 350.

The ovum of a mammal begins its growth period with a single layer of nurse cells. As the growth proceeds, these increase by mitotic divisions to a double or many layered covering. Later, the outer part of the covering becomes separated from that immediately covering the ovum, except at a single point called the hilum. The space between the two layers becomes filled with a fluid that must act as an intermediate carrier for nearly all the exchanged materials.

The double or triple layer of nurse cells, covering the egg at this time, each sends a cytoplasmic process down to the egg membrane. This egg membrane is moderately thick, but seems to be of a soft consistency.

No radial canals are shown by which the processes might pass in, or through which the yolk substance might pass (Fig. 425).

We may conclude that the yolk passes in as a fluid, or that there are canals which are too fine to be detected.

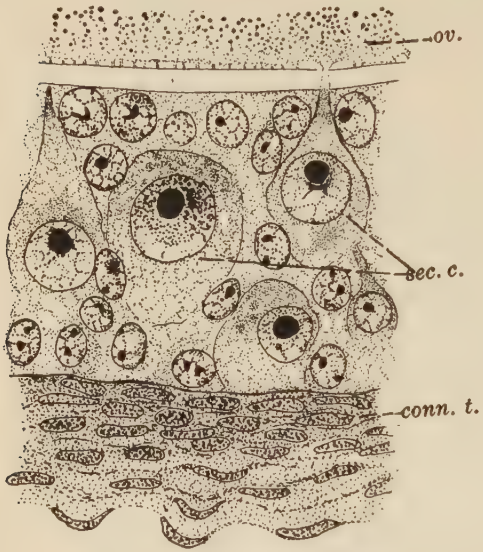

FIG. 424. — Part of the multiple layer of nurse cells in the egg-follicle of the half-grown ovum of a water snake, *Natrix sipedon*. One of the four large secreting cells (*sec.c.*) shows a process extending through the cell wall of the egg. *ov.*, edge of ovum; *conn.t.*, connective-tissue layer of follicle; *sec.c.*, secreting nurse cells. × 720.

The growing ovum often, but not always, possesses another structure which is but little understood, but which appears to be concerned with its yolk accumulation. This is a body in the cytoplasm which is known as the *yolk nucleus*. Its most characteristic appearance, perhaps, is in some of the fishes, as, for example, in **the yolk nucleus of** *Lophius*, **the angler**, where it can be seen in the egg a third grown, as a denser, darker-staining mass in the middle cytoplasm (Fig. 426).

It has been described in another characteristic form by Calkins in the earthworm, Van Bambeke in a spider, and Crampton in an ascidian. In this last type it first appears as an entire or partial ring of the dark-staining material encircling the nucleus. This ring retreats peripherally, at the same time undergoing a disintegration into smaller bodies or even into diffused granules. It finally becomes distributed throughout the cytoplasm and thus is lost as a visible, structural feature of the cell. Figure 427 is a series of three stages as figured by Van Bambeke in a spider, *Pholcus*.

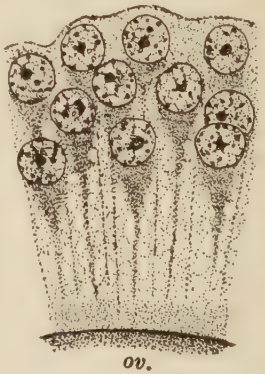

FIG 425.—Portion of the nurse-cell layer of the egg-follicle of a cat. *ov.*, edge of ovum. × 870.

A centrosome has been described as occurring in young, resting ova as well as those seen during divisions. Munson has described a peculiar body, in the ovum of *Limulus*, which has the appearance of either a centrosome or a nebenkern or a yolk nucleus.

In turning once more to the important structural changes which take place in the nucleus of the growing ovum, we find ourselves confronting

the most interesting and difficult subject in cytology. Although its essential features have been already described in the reduction of male reproductive cells, we shall outline it again as seen in the female cells, following this outline by several concrete descriptions, and finally by some account of the union of the male and female gametes to form the *oösperm* or *zygote*.

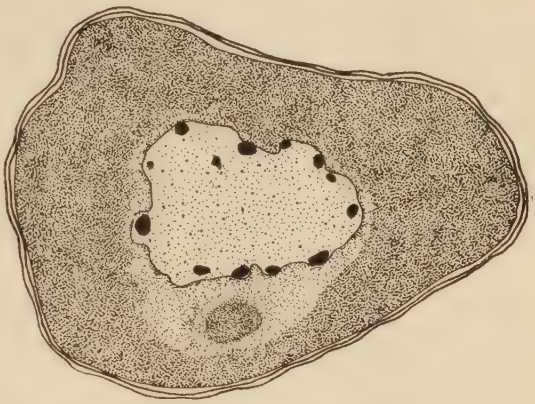

FIG. 426. — Young ovum of the angler, *Lophius piscatorius*. Shows the yolk body beneath the nucleus. × 500.

The young female reproductive cell, before it begins its growth, is known as an *oögonium*. It possesses the same number of chromatic units or chromosomes that are characteristic of the somatic cells of the species. The beginning of maturation (which is a comparatively rapid process occurring during the breeding season) is marked by a gathering of the chromatin into a closely reticular mass that lies at one side of the nucleus. This process was formerly known as *synapsis* but, as synapsis is now used as a term to designate another process which may take place during this closely reticular stage or before it (usually during the telophase of the last oögonial division), the term *synizesis* has been used to designate the close reticulum.

Upon emerging from synizesis, the oögonium begins a quick period of growth or yolk accumulation, by the means already described, and

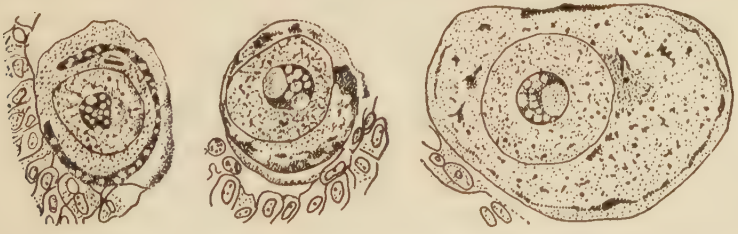

FIG. 427. — Three stages in the growth period of the egg of *Pholcus*. The yolk body appears as a ring about the nucleus, and swells and disintegrates as the yolk accumulates. (From WILSON after VAN BAMBEKE.)

then becomes an *oöcyte of the first order*. It now forms its chromosomes for the two divisions known as the reduction divisions. If the somatic number of chromosomes during a division is 12, we shall find that the

primary oöcyte has but 6 chromatin masses, which are larger, however, than usual, and in some animals can be seen to consist of four portions each. In this latter case they are called the *tetrads*.

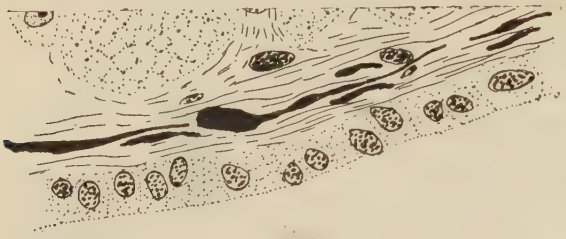

Fig. 428. — *Asterias Forbesii*. Region of young ovary from which the reproductive cells originate. × 1500. (Drawn by H. E. Jordan.)

A mitotic division now takes place in which the tetrads are split in two, and each daughter cell receives 6 *dyads*. The two resulting cells are known as *oöcytes of the second order*, and they at once proceed to perform another mitotic division without, meanwhile, re-forming the nucleus. This second division results in the dyads being pulled apart and each of the four resulting cells getting 6 *monads*, or 6 *chromosomes*, as we must term them. This is one half the somatic number. One of the 4 cells is now a matured ovum.

These equal divisions of the nuclear elements were not followed by equal divisions of the cell body and its load of yolk. When the primary oöcyte divided, one secondary oöcyte took practically all the cytoplasm, leaving its sister cell to appear as a tiny mass of nuclear substance which is discharged from the ovum. This smaller secondary oöcyte is called the *first polar body*. In the second reduction division the same thing is repeated, and one of the resulting ova is discharged (divided) from its sister cell as the tiny *second polar body*, while the first polar body often makes an attempt at division which results in there being three polar bodies attached to an ovum instead of two.

This process, especially its latter part, can be very easily traced in the growing and maturing ova of a starfish, *Asterias Forbesii*. At an early age the reproductive cells of this animal are situated in the epithelium lining a compound tubular organ, which is the ovary. They are exceedingly small and can only be detected among the somatic cells when they begin to grow in size for the maturation process. Figure 428 shows them just before this occurs. It occurs principally during a few spring and summer months in *Asterias Forbesii*, but goes on to some extent during the whole year. At this time the ova begin to enlarge and

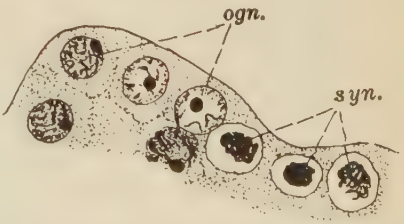

Fig. 429. — *Asterias Forbesii*. Group of young oögonia (*ogn.*). Three of the oögonia are undergoing synizesis (*syn.*). × 1500. (Drawn by H. E. Jordan.)

appear to go through the various processes in groups which may be likened to the sperm columns of the testis. The cells are exceedingly small during the early stages, so small that they can hardly be seen. They are first recognizable as reproductive cells by the large black nucleolus, and shortly after the outline of the expanding nucleus can be seen. When they are between two and three microns in diameter, a cytoplasmic body becomes apparent; the chromatin appears as a delicate spireme, and they are ready to begin maturation (Fig. 429, *og.*). No mitosis is observable which can be interpreted as an oögonial mitosis.

The cells enlarge, and the chromatin becomes a delicate thread (Fig. 429, *ogn.*). It afterward grows stouter and gathers closely around the nucleolus, which is of some size by this time. This is the beginning of *synizesis* or the contraction stage, and presently the nucleus is completely

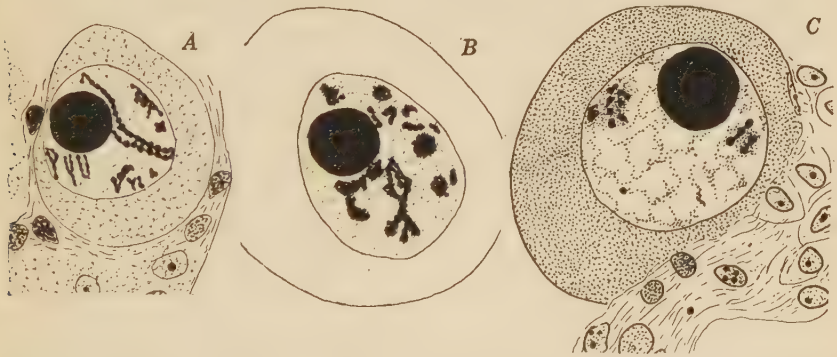

FIG. 430. — *Asterias Forbesii.* Stages of the primary oöcyte at early growth period. The nucleus grows and yolk is accumulated. The chromatin thread breaks into chromosomes, which become reduced in size. × 1500. (Drawn by H. E. JORDAN.)

hidden by the crowding together of the chromatin masses (Fig. 429, *Syn.*).

This stage does not last long, as is evidenced by the rarity with which it occurs in any given section, notwithstanding the great number of ova which are formed. More common are the stages which show the skein of chromatin relaxed. The whole cell is very rapidly growing at this time, and its size corresponds fairly well with the changes that take place in the chromatin. The skein or spireme enlarges with the widening nucleus and becomes thicker. It is double, and its strands are granular. Figure 430, *A*, shows this stage just as the spireme has begun to divide into a number of pieces. The many small pieces shown in the figure are largely the result of an artificial cutting and breaking of the chromatin in the process of sectioning.

In Figure 430, *B*, we see the portions of the chromatin spireme thickened and shortened. They present, also, a mossy appearance at this

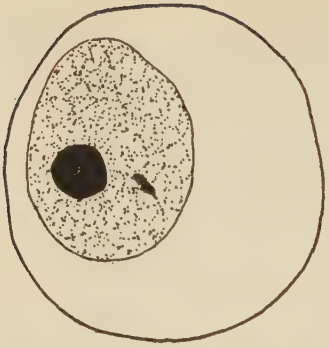

FIG. 431. — *Asterias Forbesii.* Fully grown ovum (primary oöcyte), showing large chromatic nucleolus and smaller, dark staining group of chromosomes. Lower magnification than preceding figure. × 500. (Drawn by H. E. JORDAN.)

time. This stage appears very regularly in the course of development of all the ova. The chromatin portions begin at this time to decrease in size, and soon they come to lie in one, two, or even three small groups near the periphery of the nucleus as in Figure 430, *C*. They will now be called *chromosomes*, because they are supposed to be the individual chromosomes, small and condensed in form, which afterward take part in the maturation divisions. When seen to the best advantage, they appear as tiny, bi-lobed bodies lying side by side.

The ovum, shortly after this, attains its full size, as seen in Figure 431, and the chromosomes now appear as a very small mass indeed, and are easily overlooked in the large nucleus, especially in unfavorably stained specimens. They seem to lie in almost any part of the nucleus, although they are oftenest next to some part of the nuclear membrane. In order to determine their presence and number, one must study a complete series of sections of any particular egg nucleus. Sometimes they are attached to the nucleolus and sometimes are farthest from it. As has been said, they may form one or more groups. The figure shows them in a single group.

The egg is now ready for the maturation divisions. The nucleus has become situated near the periphery of the ovum, and the ovum is ready at any time, upon a mus-

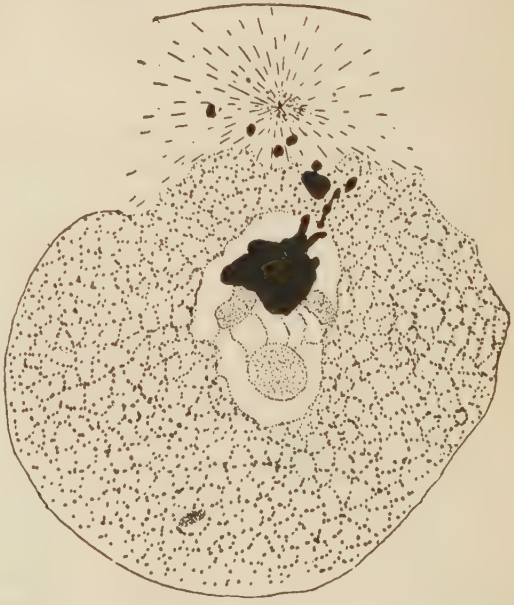

FIG. 432. — *Asterias Forbesii.* Portion of an ovum just beginning the first reduction division. Chromatin leaving the plastin ground-substance of the nucleolus and being added to the chromosomes near the forming spindle. × 1500. (Drawn by H. E. JORDAN.)

cular pressure or the crowding of the ova behind it, to be passed out of the animal's body into the surrounding water, where there is a large probability of its being near the spermatozoa which have been similarly deposited by neighboring males.

The sea water influences the ovum to go through the maturation divisions. The nucleolus and the chromosome groups are usually to be found at this time nearest the distal edge of the eccentric nucleus. In the most ordinary cases the nucleolus begins to show an irregular outline, the distal part of the nuclear membrane begins to undulate and shrivel, and a radiating figure of achromatic material, the aster, appears in the cytoplasm just distal of the nucleus (Fig. 432). In fact, it is the astral rays, combined probably with some solvent agency, which seem to cause the nuclear membrane to give before them. Presently it can be seen that the many chromosomes have separated somewhat and moved up to the aster. This latter has divided and separated, leaving a set of connecting fibrils, the spindle fibrils, which extend between the daughter asters.

As the chromosomes move up into the area between the separated asters, the nucleolus begins to discharge its chromatin as a series of irregular, semi-fluid lumps which leave behind them the plastin body that held them during the growth period (see Fig. 432). This plastin remnant does not stain deeply, and after becoming irregular it breaks up and disappears. Meanwhile, the chromatin which left it is separated into smaller granules, and part of these appear to act as a source of nutriment to the chromosomes, which increase considerably in size at this time. Part of this chromatin is distributed through the rest of the nucleus, which has now lost its membrane and appears as a larger, more granular and darker-staining area, the "residual substance," in the cytoplasm.

Often the passing of chromatic material from nucleolus to chromosomes begins to take place before the other maturation phenomena have begun. This is most apt to be seen in cases where the chromosome masses and nucleolus are at some distance from the point at which the spindle is formed. Figure 433 shows such a case and also shows how the chromatin passes out of the nucleolus in a fine, granular stream. Part of the plastin ground substance has been left free by the chromatin in this instance.

Shortly after such a figure as 432 the spindle is formed, and the chromosomes, at first widely scattered, begin to be drawn into a fairly regular equatorial plate (Fig. 434, *A*). They have already begun to divide by a longitudinal division before the plate is actually formed. Shortly after this the figure appears as in Figure 434, *B*, where some of the chromosomes have already divided, and the others are soon to follow. They

are bi-lobed, and split longitudinally into two equal halves. The split appears first at one end, and the divided ends are opened into a V-shaped figure, and then pulled apart until the V is straightened out into a line. At this point they appear as a rod with three lumps on it, one on each end and a larger one in the middle. The break now comes in the middle of the central knob, and the bi-lobed daughter chromosomes move apart toward their respective poles (Fig. 434, *C*).

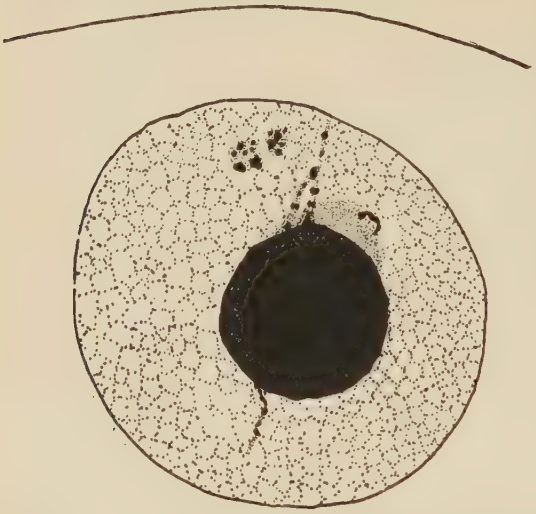

FIG. 433. — *Asterias Forbesii.* A slightly younger ovum than the preceding to show the streaming out of chromatin to a distant group of chromosomes. × 1500. (Drawn by H. E. JORDAN.)

In Figure 434, *D*, may be seen a late telophase of this division, and here it will be noticed that the distal end of the figure has emerged from the surface of the ovum, carrying a little cytoplasm with it. This is the *first polar body.*

FIG. 434. — *Asterias Forbesii.* Four stages in the first reduction division. × 1500. (Drawn by H. E. Jordan.)

Almost before the first polar body is completely separated, the chromosomes in the ovum begin to form a new equatorial plate on a new spindle derived from the remaining portion of the first spindle. Figure

435, *A*, shows such a new spindle with the chromosomes at metaphase. The bi-lobed chromosomes are again divided longitudinally into daughter chromosomes, which are also bi-lobed. Figure 435, *B*, shows a telophase of such a figure which soon throws off a *second polar body* exactly as the first figure did its first polar body (Fig. 435, *C*). In this case the first polar body is apparently making an abortive attempt to divide. Shortly after this the egg chromosomes expand into vesicles which fuse to form the female pronucleus.

Figure 435, *D*, shows the two polar bodies completed and the egg chromatin forming a nuclear membrane. In this case the first polar body has made no attempt to divide. The egg aster still persists, and the whole figure appears as an almost perfect resting nucleus with a centrosome.

A remarkable point in the whole growth period of these eggs is the lack of well-developed nurse cells. The follicle cells show only a very

FIG. 435. — *Asterias Forbesii*. Four stages to show the second reduction division and the formation of the female pronucleus (*D*). (Drawn by H. E. JORDAN.)

thin layer, complete, but so delicate that they do not seem to have the power of preparing the great quantity of yolk that the ova acquire. A possible explanation of this condition is the fact that the ovaries lie bathed in the general blood supply, and that this has such free access to the ova that they can absorb sufficient food material directly from its body. This presupposes that the blood is very rich in a food supply which needs but little elaboration to become yolk.

When the egg has matured, and is brought into the proximity of sperm, the spermatozoa are influenced by its presence to direct themselves toward it, and to make energetic swimming efforts to reach it. Upon reaching it, the first spermatozoön forces its way through the thick zona radiata at the *micropyle*, a small opening which is there for that purpose. As it draws near the ovum the latter responds to its first contact by a lifting of the cytoplasm at that point in the form of a cone, into which the sperm passes. It continues to move into the cytoplasm of the ovum, but leaves its tail behind at the surface. This surface now becomes covered with a delicate membrane, the *vitelline membrane*,

which probably prevents any more spermatozoa from entering. Figure 436 shows a spermatozoön in the ovum and another vainly attempting to enter. Sometimes two or more do get in with abnormal results.

FIG. 436. — *Asterias Forbesii.* One spermatozoön entered into ovum; second vainly attempting an entrance. × 1500. (Drawn by H. E. JORDAN.)

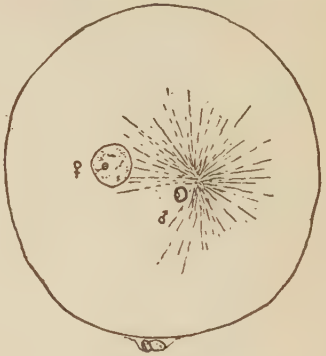

FIG. 437. — *Asterias Forbesii.* Sperm head, now becoming male pronucleus, approaching the female pronucleus. × 440. (Drawn by H. E. JORDAN.)

The use of some chemicals results in the postponing of the membrane formation, and this almost always leads to such a *polyspermic fertilization.*

As the sperm head, with its middle piece attached behind, advances toward the egg nucleus, it rotates so that the middle piece is in front. The cytoplasm through which it has passed, spreads out behind it in a widening "wake" of somewhat different staining power from its original condition. This is called the "entrance funnel." The sperm head now begins to enlarge, and a centrosome, arising from intimate connection with the middle piece, acquires rays which originate in and grow out into the cytoplasm (Fig. 437). These rays constitute the future dynamic apparatus for cell division. By the time the sperm head has arrived next to the egg nucleus, it has become enlarged and has opened out its chromatin pattern to form a sperm nucleus which is but little smaller than the egg nucleus (Fig. 438). *Asterias* thus differs from some other echinoderms, as *Toxopneustes*, in which the sperm head does not open up much until it has joined the egg nucleus. The process of union is

FIG. 438. — *Asterias Forbesii.* Apposition of the male and female pronuclei. × 440. (Drawn by H. E. JORDAN.)

one of close apposition, and the chromosomes of each, 18 in number, remain independent of each other for a long time after, forming the 36 chromosomes of the regular starfish cells. They each divide in subsequent divisions, so that every descendant has 36 chromosomes, and each of these 36 was derived from one, and a different one, of the original 36 in the newly fertilized ovum or oösperm. Later, when some of the descendants have become the male or female reproductive cells of the young animal, and they are beginning to mature, they will at last unite the paternal and maternal chromosomes into 18 bivalent chromosomes, which are the *tetrads* of maturation. This process is called *synapsis*, and sometimes occurs during synizesis or contraction. It has been seen in a few cases, in some Hemiptera, where it is described by Wilson, McClung, and others.

A study of the mammal ovum will yield a fuller account of its very early development than that of the starfish does, and therefore will serve as a concrete example of those first stages. Like the male reproductive cells of the skate, the female cells of the mammal may first be seen in the outer part of the *germinal ridges*, two longitudinal thickenings of the embryonic body-cavity wall of the body cavity. Later, these folds become developed into the ovaries, two separate bodies of mesodermal cells, each covered with a mesothelium. The reproductive cells appear as *primordial egg cells*, in or just under the mesothelium, which is a part of the peritoneum reflected over the ovary.

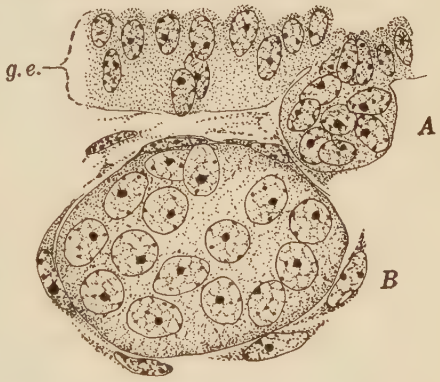

FIG. 439. — Two young egg tubules near the surface of the ovary in a kitten. *g.e.*, germinal epithelium. *A*, youngest egg tubule. *B*, older tubule. × 1000.

At a very early date (before birth in many mammals), all the reproductive cells which are going to pursue a further development are drawn down in groups into the body of the ovary. These groups we shall term *egg tubules* in general. Figure 439, *A*, shows such a group, or tubule, just below the germinal epithelium in the **ovary of an embryo of the cat**, in which we can most easily trace the early stages in the history of the mammal ovum. These cells are young oögonia, and they move inward from the epithelium, developing as they pass toward the inner part of the ovary. This movement is continued until there is a thick, cortical layer of young ova a short distance under the epithelium and extending about a third of the diameter of the ovary inward.

When first seen in this embryonic material the reproductive cells are oögonia of exceedingly small size, smaller than the epithelial cells, and they appear to originate from the epithelium or else directly under it, probably the former. They form the groups which we have called the tubules, even before they have left the surface. In other mammals they have been described as carrying an invaginated tube of the epithelium into the ovary with them. They certainly do not do this in the cat, and when they have passed in, clear of the basement membrane, it can be seen that they are surrounded by connective tissue. These ovarian tubules have been compared to the seminiferous tubules of the male.

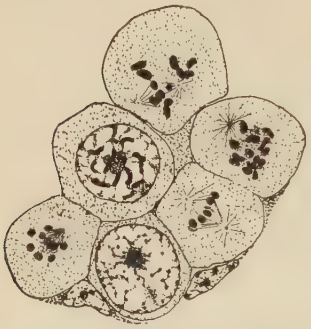

FIG. 440. — Oögonia from ovary of kitten. Multiplying by mitotic divisions. × 1000.

The nucleus of each cell is very round, and the nucleolus lies near the middle, with a reticulum of strands of linin and chromatin reaching out from it to the nuclear membrane. Cells nearest the center of the ovary in each group are somewhat the largest, and the groups inside of the first are larger than these outer ones. The figure (439, *B*) shows such a larger group which has moved into the ovary a trifle farther inside than the first.

These cells undergo a few oögonial divisions and rapidly increase in size as they move inward (Fig. 440). The nucleus increases at a greater proportional rate, and opens up its chromatin pattern. A denser area of the cytoplasm then appears in one side of the cell, and the nucleus contracts its chromatin into the synizesis stage. Figure 441 shows a part of the cells from a tubule whose cells are either in synizesis or preparing to perform it. It can be seen that a black central granule is found in the dense area of cytoplasm, and, in consequence, the structure has often been described as a centrosome. By other writers it is called a *yolk nucleus*, and considered homologous with the yolk nucleus of spiders, fishes, and other mammals. It per-

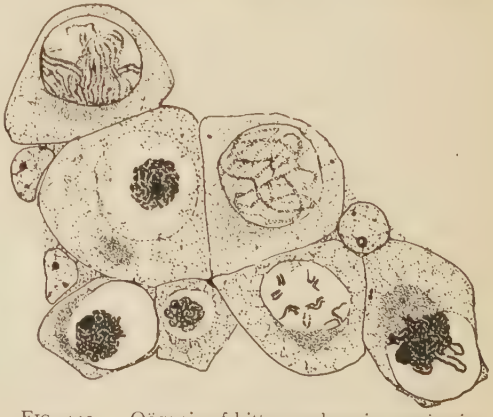

FIG. 441. — Oögonia of kitten undergoing synizesis. Yolk body present in some. × 1000.

sists from the beginning to some time after synizesis. Some of the oögonia are now seen to have not grown or otherwise changed. These are destined to become the nurse or follicle cells.

We shall not discuss in this form the debatable questions connected with the details of chromatin changes which take place before, during, and after synizesis. The figure shows that in the preceding stages the thread-like arrangement of the chromatin becomes double, and its loops appear to be connected individually with the centrosome-like body. Later, as is shown by other cells in the figure, the thread breaks up into shorter, rod-like bits which are still double. The height of the contraction stage is well shown by two of the other cells in Figure 441. All these closely successive stages form a layer, just inside of the young

FIG. 442. — Pre-oöcytes of cat. × 1000. (Compare with Fig. 1, which is the fully grown, first oöcyte ready for the reduction divisions.)

oögonia, and farther still inside we may see other stages which show what happens when synizesis is past.

These later stages show several histological differences. The tubules are being divided into smaller groups by the growth of connective-tissue septa, and it can here be seen that some of the original tubule cells which did not undergo synizesis have begun to divide and surround the young ovum, which must now be called a *pre-oöcyte*. This name is necessary to distinguish it both from the oögonium before synizesis, and especially from the later primary oöcyte which goes through the first maturation division.

The pre-oöcytes have all been formed at about the time of birth in the cat, and constitute a layer just under the surface of the ovary. This layer remains here during the greater part of the cat's life, the majority of its cells never changing, while from time to time some of them move a little farther down into the stroma of the ovary and begin a rapid growth period which ends in maturation and discharge. Figure 442 shows several cells from this layer in an adult cat. Among the pre-oöcytes

are still to be found the unchanged oögonia, now become nurse cells. The pre-oöcytes occur either singly or in groups of two and three or more. When another ovum is to be matured, one of the single cells or one of the groups moves down and becomes a primitive follicle. If more than one pre-oöcyte is in this primitive follicle, one of them develops at the expense of the others, and it is rare to find a follicle containing more than one ovum at maturity. In some other mammals very different histological conditions obtain during this history, especially in such as have no great mass of connective tissue in the ovary.

FIG. 443. — Prophase of the first maturation division in a mouse ovum. (After SOBOTTA.)

The development of the follicle has already been spoken of. The nurse cells form a single layer around the ovum and then stratify to form a double layer, which continues to multiply its cells by mitosis until there is a thick multicellular layer.

Finally, the layer which is directly around the ovum splits concentrically from the layer which lines the connective-tissue capsule of the follicle, and the two become separate except at one point, called the *hilum*. When the ovum is ripe, the capsule and the outer wall of the ovary break, and the egg is discharged into the body cavity, whence it passes into the oviduct and down to the uterus.

From the time that it leaves the layer of pre-oöcytes, the reproductive cell undergoes a rapid growth, and at or about the time it is discharged from the mature follicle, it undergoes the two maturation divisions. The cat does not easily exhibit this part of the process, and so we shall turn to rodent material to examine these in the mammal.

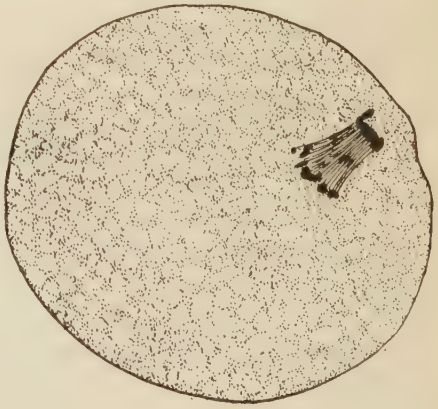

FIG. 444. — Anaphase of first maturation division in the muskrat, *Fiber zibethecus*. × 750.

The first maturation spindle in the maturing ovum of the mouse is composed of distinct fibers without astral rays. Despite Sobotta's claim that the spindle fibers do not converge but tend to lie parallel, as straight lines from pole

to pole, the fibers do, according to Lams and Doorme, and Kirkham, converge to points at which lie centrosome-like granules.

The first maturation spindle bears about 12 reduction chromosomes. The number of chromosomes is yet a matter of dispute. Lams and Doorme have counted in two cases 12, and state the number as varying from 12 to 15. Likewise Kirkham is inclined to make his count 12. Sobotta gave the number as 12.

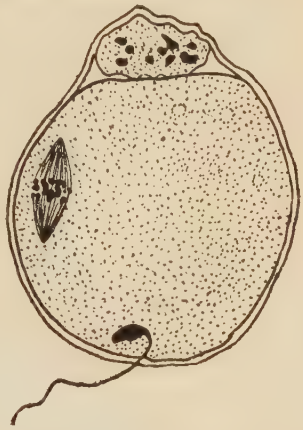

FIG. 445. — Ovum of mouse, showing first polar body, second polar spindle, and entering spermatogoön. (After LAMS and DOORME.)

The spindle at this stage usually lies parallel to the surface of the ovum (Fig. 443); after metaphase the chromosomes lie at the two ends of the spindle, which has rotated so as to be at right angles to the surface. Figure 444 shows this stage in the maturation of the muskrat ovum. The spindle fibers at this stage both in the muskrat and mouse tend to be parallel straight filaments.

The first polar body is formed as a bud from the surface of ovum into which the distal end of the first maturation spindle travels. In this position the spindle is divided, at the cell plate, by the constricting of the bud to form the first polar body.

The remains of the first maturation spindle, left within the ovum, re-forms as a pointed spindle, which according to Lams and Doorme becomes about the size of the first spindle. At its equator twelve or more chromatin bodies assemble. As the second maturation spindle retreats, it also assumes a tangential position (Fig. 445).

FIG. 446. — Mature ovum of mouse, showing first and second polar bodies, female pronucleus (*f.p.*), and male pronucleus (*m.p.*). (After LAMS and DOORME.)

The metaphase and anaphase stages of the second reduction mitosis ensue during the time that this second spindle rotates at right angles to the surface of the ovum. In a like manner a second cytoplasmic bud is formed, into which the distal half of the second maturation spindle passes to be constricted off with the separation of the second polar body.

While the second maturation mitosis is taking place, an entire sperm, according to Lams and Doorme, and Kirkham, enters the cytoplasm of the ovum.

With the complete formation of the second polar body the sperm head enlarges and becomes reticular to form the male pronucleus (Fig. 446, *m.p.*). At the same time the chromatin, and the part of the second maturation spindle which remains within the ovum, become organized into the female pronucleus (Fig. 446, *f.p.*). The blending of these two pronuclei results in the nucleus of the fertilized ovum.

Among many animal forms, most of them highly specialized, the female gives birth to some of her young or lays eggs that hatch and develop without the presence or aid of any male. This condition is common among certain insects and crustaceans as well as some other animals, and is known as *parthenogenesis*.

The young so produced are not the only offspring of their parent, for at other times certain young are derived from eggs that have been fertilized by the male cell or spermatozoön, and which develop as usual.

Sometimes all the parthenogenetic young are females, a state which is termed *thelytoky*. In other cases only males result from the parthenogenetic process, and this condition is termed *arrenotoky*. When both males and females are produced, the process is known as *amphotoky*.

The cytological question which at once suggests itself is, do such offspring come from eggs, and if so, do the eggs mature and develop with half the number of chromosomes or do they secure a full complement of chromosomes in some other way?

Investigation shows that the eggs do mature; that sometimes they give off two polar bodies and in other cases only one. As both these cases occur in *Artemia*, the brine shrimp, and as the parthenogenetic process has been worked out and understood in this form by Brauer, we shall use a description of this form as a concrete example.

The normal number of chromosomes in the cells of this animal is 168, and that number exists in the earlier stages of the reproductive cells of parthenogenetic females. At the time of maturation, these chromosomes become arranged as 84 tetrads, and in the ensuing division these latter are separated, 84 dyads going to the first polar body and the other 84 staying in the oöcyte.

The subsequent development shows two forms. In the second type of Brauer (which we describe first because it seems more like the usual methods), the remaining 84 dyads in the egg divide, and one set of 84 of the resulting chromosomes remains in place to form the egg nucleus, while the other 84 pass to the surface to help form the second polar body.

If the egg nucleus with its half number of 84 chromatin units were to proceed to develop by cleavage, there would result an embryo and adult, all of whose nuclei had but one half of the proper number of chromosomes. Before development begins, however, the second polar body returns and unites with the egg nucleus, and then development proceeds

as though it were a male element that had been added to the egg nucleus instead of its own sister cell, the second polar body. This process is shown in Figure 447, which is copied from Brauer.

The other type of maturation, Brauer's first type, may be said to be like the one that we have just described except that the second polar body

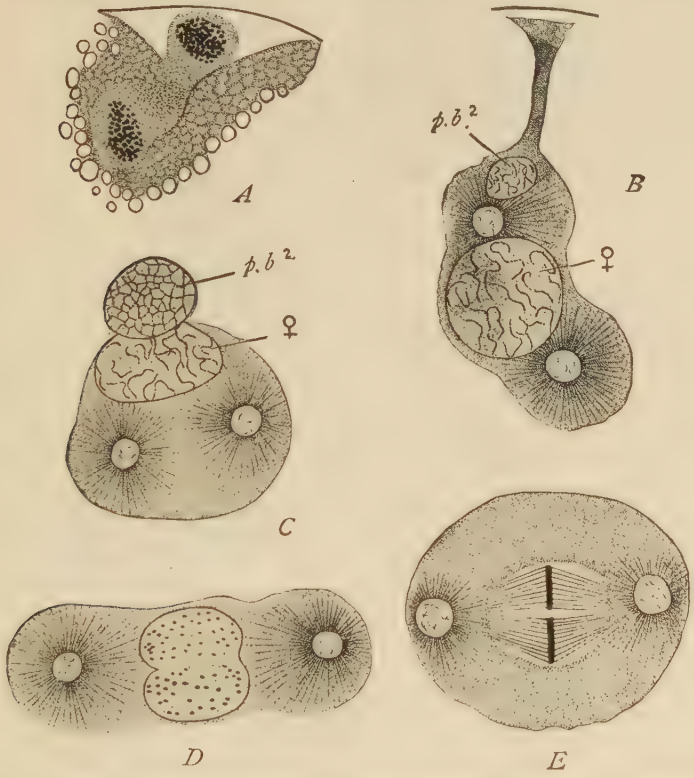

FIG. 447. — *Artemia salina*. Several stages in the maturation of the kind of parthenogenetic egg that gives off two polar bodies. *A*, formation of first polar body (*p.b.*²); 84 dyads in this polar body and 84 others in remaining nucleus; *B*, second division of the egg chromatin, which results in a second polar body with 84 single chromosomes and an egg nucleus with 84 chromosomes; *B*, return of the second polar body (*p.b.*²); *C*, *D*, *E*, three stages in the union of the second polar body and in the first cleavage division of the completed zygote. (From WILSON after A. BRAUER.)

is never formed, the chromosomes that formed it in the preceding example merely remaining in place. In fact, it seems a useless proceeding for this second maturation cleavage to take place in any event, when the chromosomes are to immediately return and again join those from which they had been separated but shortly before. The latter method, therefore, seems to be the ultimate specialization, while Brauer's second method is a more primitive process. Figure 448, from the same source as 447, shows some of the principal stages in this process.

Technic. — The technic used in preparing the female reproductive cells for study differs very markedly from that of the preceding part. This is owing solely to the great mass of yolk which most ova contain, as well as to the membranes of several kinds with which they are usually

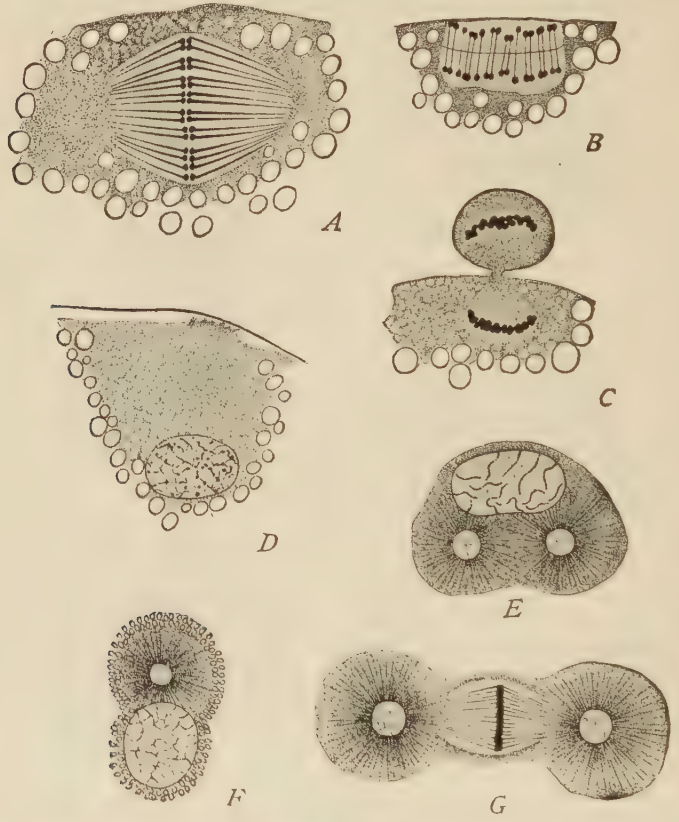

FIG. 448. — *Artemia salina*. Several stages in the maturation of the kind of parthenogenetic egg which gives off but one polar body. *A*, first polar spindle with 84 *tetrad* chromosomes; *B*, *C*, formation of first polar body; *D*, egg nucleus, formed from the remaining *dyad* chromosomes (84); *E*, *F*, *G*, formation of first cleavage figure. (From WILSON after A. BRAUER.)

covered. For this reason, Flemming's fluid is rarely used, and if a general fixative were demanded, we should first mention sublimate-acetic (a saturated, watery solution of corrosive sublimate containing five per cent of glacial acetic acid). Even this has to be put aside in many cases where the yolk becomes too brittle, and its place is taken in most cases by a mixture of picric acid and acetic acid in several combinations. In some few cases the yolk can be softened or even dissolved. For instance, by fixing teleost fish eggs in corrosive sublimate only long enough to kill the embryo, and then completing the process in five per cent formalin,

the yolk is usually softened and may be cut. Or, by fixing the ova of *Loligo* in osmic acid until the outer blastoderm is killed, and then removing to a very dilute solution of chromic acid, the yolk may be completely dissolved.

In refractory cases, celloidin combined with paraffin is used, and for some ova celloidin alone, which makes it hard to cut thin enough sections or to secure a good series. Many studies have been performed by smear preparations with the same limitations as have been mentioned in the case of the male reproductive cells. A very important method is to study some ova in the living state or to slice or tease off the blastoderm and mount it whole.

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CHAPTER XXII

MALE AND FEMALE NIDAMENTAL TISSUES

By the *nidamental tissues* is meant all those animal tissues which are used to secrete or form coverings of fluid or solid material in which the ripe reproductive elements are to be transported or protected upon leaving the gonads. Many forms show no development of such organs, and the eggs and sperm are cast out at random into the surrounding water. The sea urchin and starfish show an example of this condition. The degree of the individual's specialization and organization does not seem to affect the development of these structures, as can be noticed when we recall that most of the highly organized and specialized teleost fish deposit their ova and sperm much as the echinoderms do. Also many low and simply organized creatures have elaborate nidamental organs, as the flatworms and others. We shall, therefore, in our discussion, pay little attention to the systematic position of our example, merely indicating the conditions under which it lives and which make these structures a benefit.

The use of fluid as a carrying body is one that greatly aids the proper placing of the reproductive cells. While this is used in the discharge of some eggs, it is of especial use, and a necessity to, the transportation of the very small spermatozoa.

Most eggs are carried or aided in their passage from the body by: *first*, some sort of follicle liquor which is secreted by the cells of the *membrana granulosa*, a region of differentiated nurse cells in the ovary of man and other vertebrates; and, *second*, by the general cœlomic fluids of the body cavity which occur in all forms in which the gonads rupture into this space.

Likewise, the spermatozoa are invariably borne in a liquid that is secreted or in some way produced in the seminal lobule. This liquid is reënforced in some cases by the products of certain accessory glands which pour out a heavy fluid that is specially adapted in consistency to carry and discharge the sperm, and is also fitted by its composition to nourish and keep them in health and activity for long periods.

The prostate gland of the mammals is a specific example of such an accessory structure. Figure 449 shows a section of this gland taken

from man. This section shows a compound alveolo-tubular gland with a simple columnar secreting epithelium. The cells of this layer are clear and secrete continuously without a degeneration and renewal of their cytoplasm. The secretion is not plainly visible at any stage of its elaboration, and no trophospongia have been described. The nucleus is round and placed close to the proximal end. Many concretions are found in the lumen of this gland.

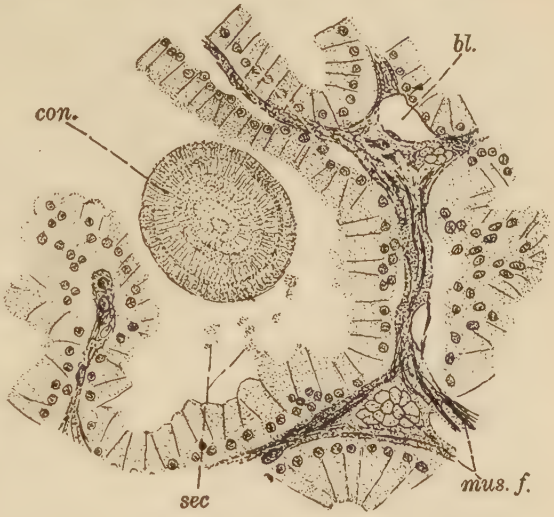

FIG. 449. — Part of several acini of the human prostate gland. *con.*, concretion; *mus. f.*, smooth muscle fibers; *bl.*, blood channel; *sec.*, coagulated secretion. (After LEWIS, in "Stöhr's Text-book of Histology.")

Another form of male-carrying fluid is secreted by **the spermatophoral glands of certain crustaceans**, as the lobster and the crayfish. This fluid is secreted by the walls of the sperm ducts (Fig. 450), and it not only serves as a vehicle to carry the mass of sperm out of the male organs, but it also forms a semifluid covering around them and attaches itself to a receiving plate on the female body and hardens, preserving the life of the spermatozoa for months or even years until they are needed to fertilize the eggs. When this time comes, the female surface secretes a fluid which softens the hardened sperm fluid and brings the dormant spermatozoa back to activity. Such a package is known as a *spermatotheca*.

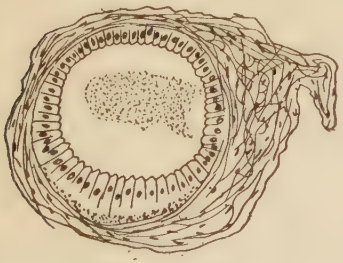

FIG. 450. — Transection of the seminal passage of a lobster, *Homarus*. Shows the epithelium that secretes the heavy, sticky, spermathecal covering for the masses of spermatozoa. (After H. HERRICK.)

Other carrying fluids for the spermatozoa are to be found in many other animal forms. A step in the organization of this apparatus may be seen in the salamander, the male of which secretes a covering for the sperm. This takes place in the thickened folds of integument which border upon the cloacal open-

ing. Some leeches also form double spermatotheca as described by Whitman.

The most complicated, exact, and highly specialized of the male nidamental-tissue products is to be seen in **the spermatophores of certain cephalopod mollusks.** This beautiful mechanism is produced in a differentiated portion of the seminal duct. This differentiated region consists of four divisions of the seminal tube. A prostate gland described in an unpublished work by L. W. Williams as a compound lamellar gland, and a glandular cæcum take part in the formation of the spermatophore. The exact manner, however, in which the structure is formed is not known. Figure 451 gives some idea of its complexity and of the delicacy of the processes by which it was formed.

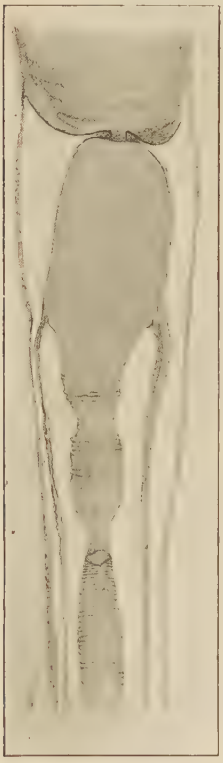

FIG. 451. — View of the central region of a spermatophore of the squid, *Loligo Pealii*. The mass of spermatozoa lie in the heavier mass in front (= above). To illustrate the exact and finished work of the male nidamental tissues of this animal. × 50.

In the female, the number, complexity, and peculiar distribution of nidamental structure is a formidable obstacle to a short and comprehensive account of them. As has been said, some comparatively few forms discharge the eggs externally in a fluid. And again, some of the highest forms which develop the ova internally, do so without the aid of any shell or fluid envelopes whatever. The follicular and cœlomic discharging fluids have already been mentioned in the remarks on that subject.

The planarian worms make cocoons in the uterus by depositing a chitinous envelope around the ovum. In some forms this cocoon has, in addition to this covering, an outer layer by which it is attached, as a stalk, to rocks, leaves, and other objects. The materials for these cocoons are secreted by the columnar epithelium that lines the uterus and ducts.

Among some other worms can be found fairly well specialized nidamental tissues. Two of these, the earthworm, *Lumbricus*, and the leech, *Pisicola*, show interesting and typical forms.

The specific cells of both of these organs are modified mucous cells, which have probably been evolved from the ordinary epithelial mucous cells, such as are to be seen in the covering epithelium of the earthworm.

The nidamental cells of *Pisicola* lie inside the body cavity, or, to be

more exact, they form two longitudinal, lateral bands just inside the longitudinal musculature of the body wall. The cells are enormous in size and very roughly cubical in shape. They lie with what must be considered their proximal end against the longitudinal muscle, and, although they are packed very closely in the layer, there is always a space between them for the access of blood or cœlomic fluid. A fine, thin connective tissue with small, highly differentiated cells surrounds the cell body (Fig. 452).

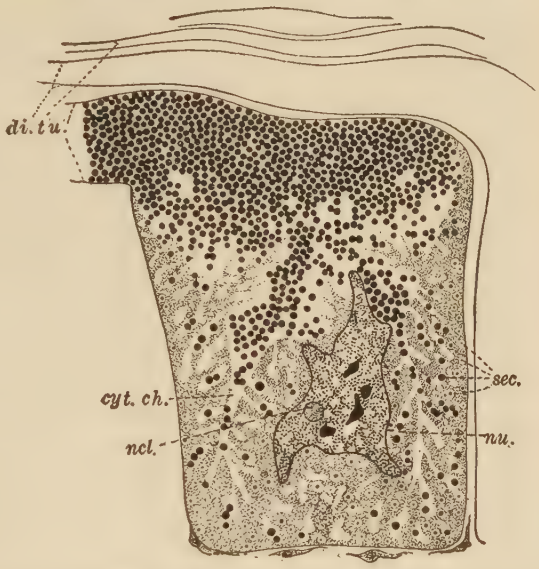

FIG. 452. — Gland cell from leech, *Pisicola*. *sec.*, secreted materials in various stages of elaboration; *ncl.*, nucleolus; *nu.*, nucleus; *cyt.ch.*, cytoplasmic channels containing and delivering the secretion granules to the large distal vacuole; *di.tu.*, discharging tubes of this and two other cells.

The nucleus lies somewhat proximal, and is very irregular in shape. It is drawn out at a number of points on its surface into irregular and thin, pointed processes. The chromatin appears as a large number of granules, and there are evidently other bodies in it — an achromatic nucleolus and some roughly rod-like and pointed chromatin bodies.

The cytoplasm is of most interest. Proximally, it is rather more homogeneous, but, distally, in the cell body its place is almost entirely taken by the great secretion vacuole. Reaching back from this vacuole are a series of branching channels or trophospongia, and in the cytoplasm which borders on the channels can be seen secretion granules in all stages of formation. When completed, these granules are discharged into the channels and carried down into the large secretion chamber or vacuole.

This vacuole is produced distally into a long tube, which runs, together with a group of similar tubes from other cells of this kind, anteriorly to a region near the head, where all these tubes penetrate the body-wall tissues, and end externally between the columnar epithelium cells of the epidermis. The secretion is mucin, and although the cells have been pointed out as excretory cells, there is no doubt that they are mucous

cells of the epidermis, extraordinarily developed and enlarged to make a cocoon for the eggs. A considerable proportion of the body is comprised by these cells, and the expenditure of the animal's energy in secreting mucus must be large.

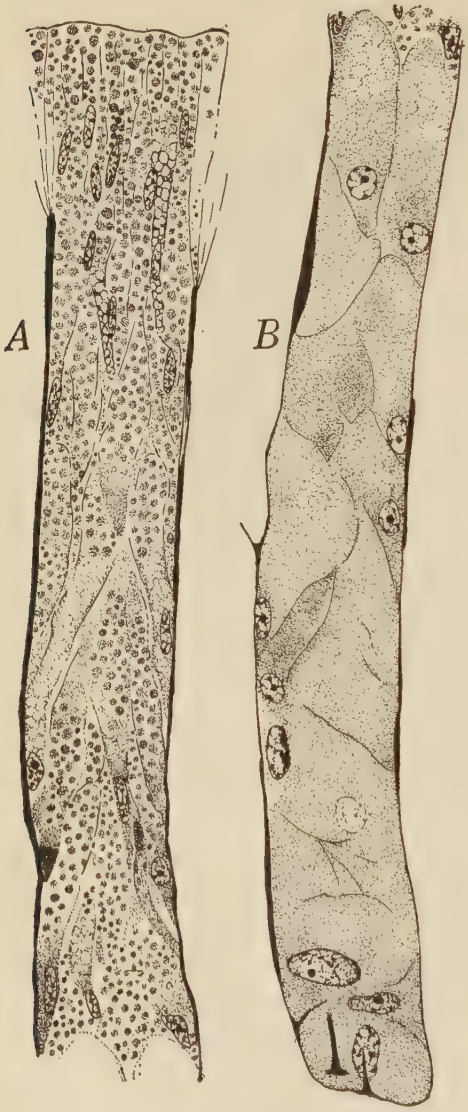

FIG. 453. — Distal (*A*) and proximal (*B*) portions of a single clitellar gland of the earthworm, *Lumbricus*. × 780.

The secretion appears in some of the cells in a dissolved state, and as solid granules in others. It is used both to make the cocoon and to keep the body covered with the slime that is always found on its surface. The making of the cocoon has never been described or seen by the writers in this species, which was taken from summer flounders on the Massachusetts coast, but it is undoubtedly done by forming a shell of mucus in the clitellar region around the body, and then, after depositing the eggs and sperm, slipping it off the body and attaching it as other leeches do.

The nidamental mucous tissues of the earthworm are to be found in an elevated, band-like ring about the anterior part of the body, the *clitellum*. Transverse sections of this region show that mucus is secreted, not from unicellular glands as it is in the rest of the animal's body, but from closely set, tubular invaginations in whose long acini can be seen the large mucus-forming cells (Fig. 453). The cells do not show a very clearly marked innate type at the bottom of the fundus. For this reason, and also because their nuclei show no degeneration process, it may be concluded that they

are permanent cells and not destroyed in the process of forming the secretion.

The real cytoplasmic body of these gland cells is hard to define on account of the large amount of secreted material with which the cells are filled. The nucleus lies proximal in the cell and is large, and usually round. Its plasmosome is larger than any other found in the tissues of *Lumbricus* except in the young ova and in some nerve cells. Toward the gland mouth and lining the exterior, the nuclei are somewhat smaller and inclined to be oval. The distal part of the cell body is bent up and extends up through the lumen toward the mouth of the gland. Together with the same parts of its fellow-cells, it fills the lumen, and one cannot determine easily where the cell ends distally. The secreted material is very clear, and, if granular, is very finely so. In the peripheral part of the gland the secretion is more coarsely granular.

That part of the epithelium which touches the exterior is entirely different in appearance. The cells are very narrow, and their nuclei are also long, narrow ovals in shape. The nucleus has a more abundant supply of chromatic material, and the plasmosome is smaller than the nucleus of the deeper cells. These cells also secrete, but the material is not so abundant.

Very highly developed female nidamental tissues are to be found among the mollusks.—They are used to make both individual and collective envelopes for the ova. One especially interesting one is to be seen in the gasteropod, *Sycotypus canaliculatus*, in which the egg case is formed in a heavy-walled, glandular part of the oviduct. The tough and membranous walls of the many egg cases, as well as the string to which they are regularly attached, are all secreted by the glands found in the walls of the oviduct, a long tube which carries the eggs to the exterior. The eggs come into the oviduct in groups of from 40 to 150, in a medium-sized specimen. Each group lies in a fold in the thick, glandular wall, and this fold forms a case around it. The glandular thickness occupies two longitudinal bands of the lining of the duct, and on one side, where they meet, is a long groove in which the string that bears the cases is formed *in situ* with the cases attached to it. The epithelium lining this groove is different from that which secretes the materials for the cases. Figure 454 is a rough diagram of one side of the groove, and a small portion of the glandular thickening on the same side.

At the bottom of the groove it is least differentiated and is a columnar epithelium with rather narrow cells and nuclei. On the lower side of the groove it begins to be thrown into folds and an occasional large "goblet cell" full of mucin granules is seen, especially on the top of the folds. This condition is exaggerated nearer the top of the groove, where the

folds of epithelium are much higher; the cells on the high curve are all provided with mucous vacuoles.

Another feature is to be noted here. Besides possessing goblet cells, the epithelium on the folds is evaginated into long tubular glands, the first of which are unicellular glands with their basal portions lying in the connective tissue beneath the epidermis. These are rapidly supplanted farther out of the groove by multicellular glands which open

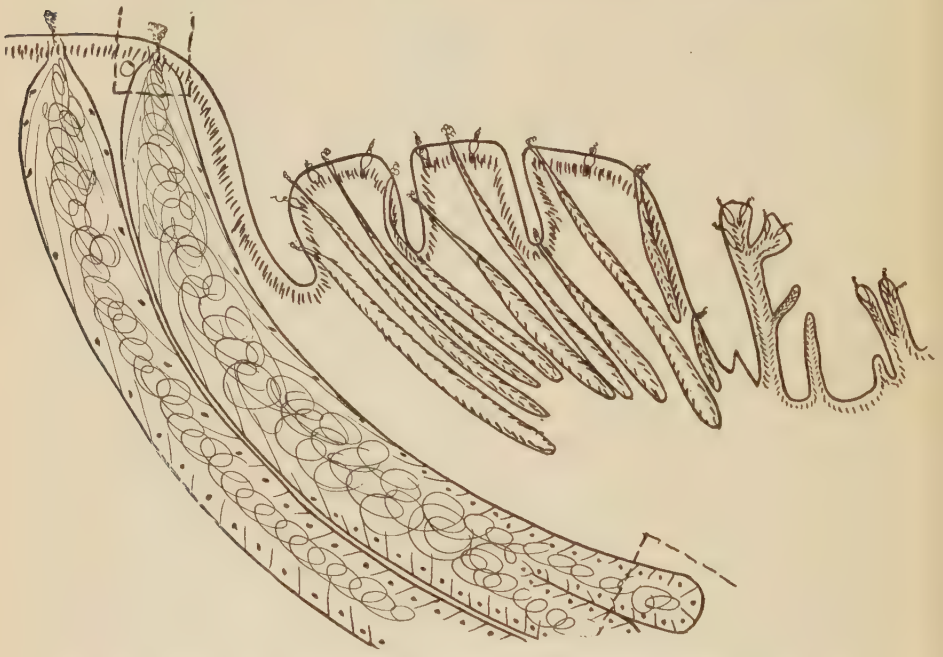

FIG. 454. — Outline sketch of one side of the groove in a transverse section of the oviduct of *Sycotypus canaliculatus*. To the right are seen the simple folds, with occasional unicellular mucous cells. In the middle the heavier folds with both unicellular and multicellular glands. To the left the very large multicellular glands with dotted outlines to mark the regions figured in the next illustration. × 50.

by narrow ducts, and whose lumen is filled with a blue staining content which still shows mucin reactions.

Out on the primary nidamental surface, the same structural conditions obtain, except that they are exaggerated, so far as the glands are concerned. These are much lengthened, and their straight, parallel bodies form a huge layer, which gives this part its swollen, whitish appearance. Two of these glands are represented to the left of Figure 454. As in the earthworm's clitellum the cells are best defined at the fundus, and farther distally their outer bodies cannot be distinguished in the mass of secretion which fills the lumen. The proximal portions of these glands

form an almost solid mass with but little connective tissue between them, and the gland-cell nuclei lie flat in the cells. On the other hand, the distal parts do not lie so close to one another, but are separated by a considerable space filled with the typical molluscan connective tissue with its coarse reticulum and many alveoli. In the figure it can be seen that blood channels occur in these spaces. The secreting cytoplasm of the gland cells shows a delicate reticulum with fine, black granules at the intersections of its strands, and large, light-blue staining granules in the meshes. These latter are probably the secretion; the first are possibly microsomes.

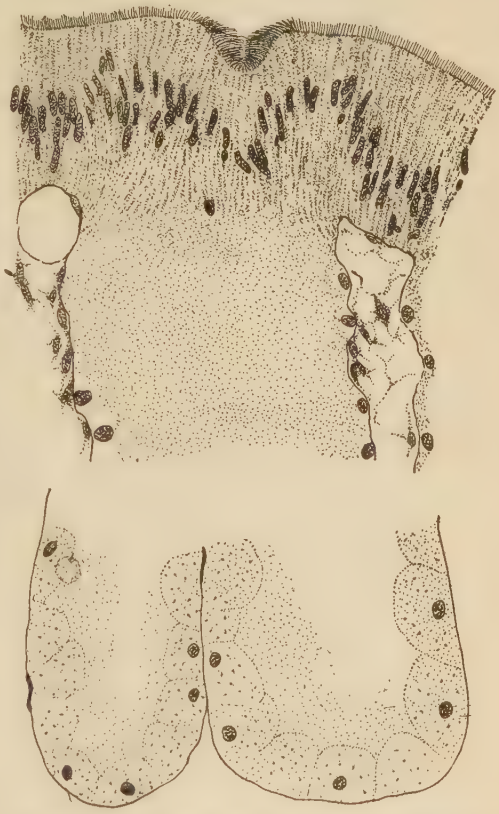

FIG. 455. — Distal and proximal extremities of a compound tubular nidamental gland from the oviduct of *Sycotypus canaliculatus*. The actual opening of the duct is small and obscure, and its location is indicated in the figure by the depression in the ciliated epithelium. × 500.

The opening of these glands is exceedingly small and hard to see, among the epithelial cells on the primary surface. Figure 455 shows the single distal part and double fundus of one of these glands as indicated by the dotted lines on Figure 454. The cells lining the surface outside of the groove show no mucin inclusions, and have very large basal granules on the marginal ends of their short, strong cilia. These cilia are used to move the eggs and egg cases, there being no peristalsis.

Among the vertebrate animals we shall examine the histological structure of the nidamental organs of: first, a *urodele amphibian* that forms two jelly coverings for its individual ova; second, a *teleost fish* that forms a single jelly covering for a great many of its eggs; third, a *selachian fish* that forms an albumen covering followed by a tough, chitinous covering for each of its eggs; and, lastly, a *bird* which forms a tough albuminous

layer, a soft albuminous layer, two membranous layers, and finally a hard outer shell for each egg.

The first type of organ mentioned will be well represented by **the oviduct of the salamander**, *Desmognathus fusca*. The specimen represented in Figure 456 was killed a short time (a few weeks) before the breeding season, and consequently the nidamental tissues were preparing for their task by an increase in the size and characters of their specific cells.

This tissue consists of a long tube with an expanded open end that lies far forward in the body cavity. From this end it takes a curving course to the point at which it empties into the cloaca. The ova enter its upper end by the action of ciliated cells, and, while on their way down its lumen, each one is invested by two coats of a jelly substance. These

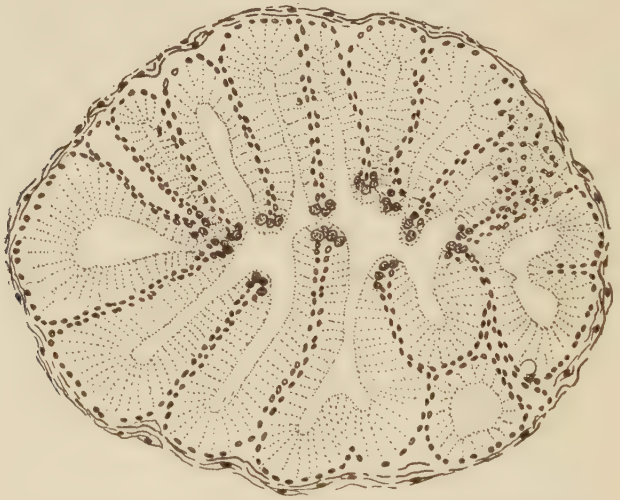

FIG. 456. — Transection of an oviduct of the salamander, *Desmognathus fusca*. × 80.

coats are not as thick as they would be in a frog or the salamander, *Amblystoma*. The outer coat is thinner and tougher than the inner. The inner layer is put on the egg in the upper part of the duct, but no structural differences are to be noted between the glands of this part and those of the point at which the tougher coat is applied. Such a difference must exist, but it may be a chemical difference or one not easily noted in the structure of the cells.

Figure 456 shows a section of the tube near its upper end, or where the first jelly layer is applied. The large lumen of the duct is closed, of course, in this specimen, owing to the fact that it was not distended either naturally or artificially by any fluids.

Opening into this lumen are a great many wide tubular glands which show a tendency to become larger or even compound at their proximal

(outer) ends. In transverse sections of the oviduct these tubular glands have been mistaken for sections at right angles to longitudinal corrugations. The right-hand part of our figure, although a transverse section, gives proof that such is not the case in this form, and that the folds in the section represent glands of a tubular variety.

The cells lining these glands form a columnar epithelium, and are intensely active in secreting the jelly substance. The secretion appears as a granular mass that fills their distal ends. The nuclei lie flattened against the proximal surface, and are very small and dense. The outer edges of the folds represent the primary surface of the duct lumen. Here the gland cells give way to a non-secreting form with a larger nucleus placed in the middle height of he cell.

The whole tube is surrounded by an inner and an outer longitudinal layer of smooth muscle. The ovum is passed through the tube by a wave-like contraction of these layers, which is called *peristalsis.*

The fish, *Pterophryne histrio*, is one of a large group, the pediculate fishes, which **lay their eggs embedded in a long ribbon-shaped plate of jelly** which floats on the sea until the eggs hatch.

This ribbon of jelly is made, not in an oviduct, for the bony fishes have no long oviduct, but by a part of the ovary itself. This organ, like that of other teleosts, is a tube-like sac formed by the union of two epithelial folds. In a mackerel and in most other members of this group the entire inner surface of this long pocket is used to produce the ova. In *Pterophryne* the sac hangs from its suspensory membrane, with its lumen closed to a straight line and its cylindrical wall thereby divided into two halves which rest against each other, face to face. One side is used to develop the ova, and this side is raised into the numerous evaginated papillæ that occur in the other teleost ovaries. The other and opposite side retains its simple epithelial structure, and the epithelium secretes the jelly materials in the form of a flat sheet, the exact length and width of the layer from which it originated. The jelly strip and the ova are produced at the same time, and their position, opposite and against each other, causes the ova to be pressed into the jelly, and then the whole mass is passed out of the ovary, one from each of the two divisions, and the jelly swells a little and floats about until the eggs develop. Figure 457 represents a fold (not a gland) of this membrane in section. The skate, *Raja erinacea*, will serve to represent the third vertebrate type to be described, in which **the ovum is covered first with a coat of jelly-like albumen, and afterward with a tough shell** of rather remarkable shape. As in the amphibian, the ovum is set free from the ovary into the body cavity and is then conducted, probably by ciliary motion, into the opening at the anterior end of the tube-shaped oviduct. This wide opening soon narrows down, and in its upper part the albumen covering is secreted

by a columnar epithelium which lines the wide lumen without any amplification whatever, as was the case in other oviducts. The cells are columnar and have both a thickened border and a line of cilia. The secretion appears as a globule in the cell body, and is discharged as mucin is.

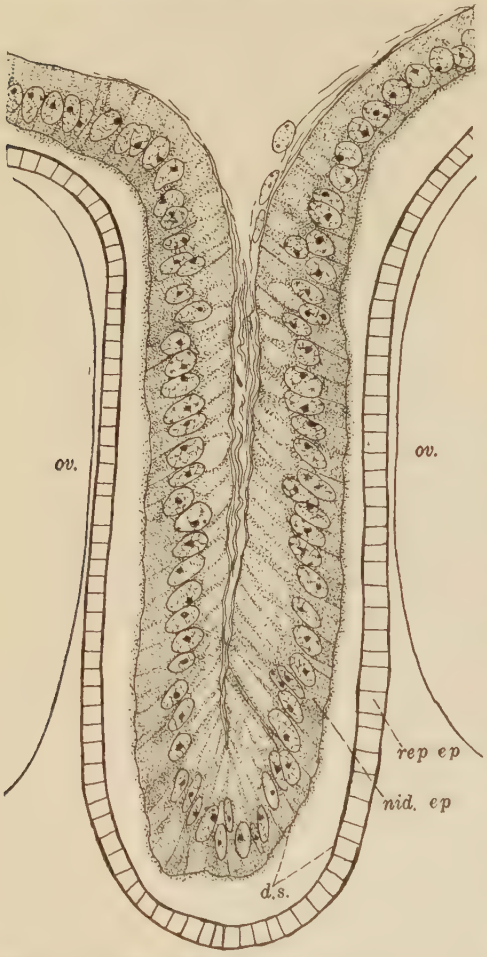

FIG. 457. — Parts of the opposed epithelia in the ovarian tube of the fish, *Pterophryne histrio; d.s.*, distal surfaces of the two epithelia; *nid.ep.*, nidamental epithelium; *rep.ep.*, reproductive epithelium in outline; *ov.*, outlines of two ova lying in follicles inside the reproductive epithelium. × 840.

A short distance below, the tube narrows somewhat, and its walls become thicker. This region is not extensive, and forms a short but prominent enlargement called the shell gland. An examination of the epithelium of this organ shows that it is lined by much the same kind of epithelium, except that the cells are much larger and the surface is thrown into shallow tubular glands whose cells have undergone no marked differentiation, and which are probably obliterated by stretching when the large egg passes through.

In the lowest part of the oviduct, the walls are much thicker, the lumen larger, and the epithelial lining is changed from the columnar form of the preceding parts to a very peculiar, distally elongate, stratified form (Fig. 458). A cell of the basal layer of this epithelium extends as a ribbon-like fiber for almost halfway out to the lumen. The outer cells are wedged in between the ends of basal cells and form a somewhat irregular distal boundary to the epithelium. Here they show signs of degeneration. The possibility remains that some of the cells extend from basement membrane through to distal surface, and that the layer is theoretically a simple epithelium. Even were this so, the practical

result of having the cell bodies with their contained nuclei at so many levels, would be to make it serve as a stratified epithelium.

The oviduct of the fowl acts very much as that of the skate did. — It differs functionally from that of the skate in producing more various and more numerous coverings for the egg. We shall compare its structure briefly with that of the skate by describing the epithelial lining of an upper and a lower region.

A section taken near the beginning (Fig. 459) shows that here the tube is lined with a columnar epithelium which is simple, but whose nuclei are placed at somewhat various heights in the cells. This gives a result which may be compared with the strange epithelium found in the skate's lower oviduct. The nuclei are found only distally in the layer, which is exceptional. The cells show no signs of secretion, which may be accounted for by the fact that the hen was not laying at the time she was killed. A section taken in the lower part of the tube shows a decided difference from the skate's corresponding tissue (Fig. 460). This region, besides its simple epithelium, is invaginated into numerous glands whose ducts and mouths are, for some unknown reason, very difficult to see. This was also the case in other nidamental glands, as the salamander and the invertebrate forms, and seems to be a characteristic of this tissue.

FIG. 458. — *Raja erinacea;* oviduct. Thick, stratified epithelium whose cells are distally elongate. × 450.

The glands are lined by a cuboidal epithelium whose nuclei are round, and whose lumen but rarely shows in our specimen. It would probably show better in a laying hen. The nuclei of the superficial cells are long and narrow; more so than in the same kind of cells from the first region.

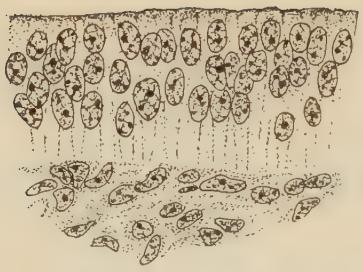

FIG. 450. — *Gallus domesticus;* upper part of oviduct. Shows a simple, tall, columnar epithelium. × 1100.

The study of organs of copulation is principally a morphological one. The histology of these structures is very generally that of mere integument muscle and other general tissues.

We shall examine one case of a tissue specifically designed to aid this process in the mammals, the erectile tissue. **The urethral portion of the human** *corpus cavernosum* will represent the tissue which is used to enlarge and make rigid the intromittent organ (Fig. 461).

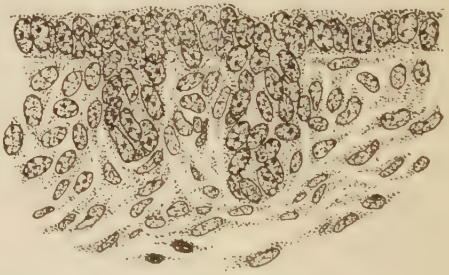

FIG. 460. — *Gallus domesticus;* lower region of oviduct with short tubular glands. × 1100.

The tissue consists of a connective tissue containing many smooth muscle cells. It is entered by arteries whose walls show peculiar elastic tissue enlargements directly under the intima. These arteries end, in the cavernous tissue, in capillaries which in turn discharge their contents into a series of thin-walled veins which are so large and so numerous that they give the tissue its name, the cavernous tissue.

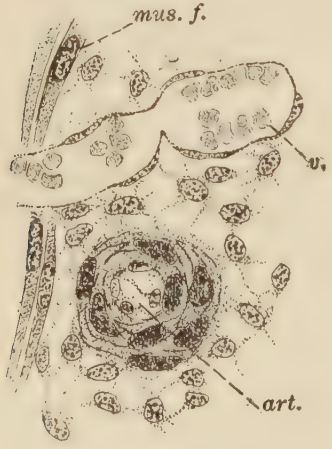

FIG. 461. — Small area from a section through the corpus cavernosum of an infant. *v.*, vein; *art.*, artery; *mus.f.*, smooth muscle fibers. × 500.

These cavernous veins are filled with blood, and the efferent veins are so compressed at the same time that this blood cannot escape. This produces the necessary rigidity of the tissue.

Technic. — The different nidamental tissues require a variety of treatment. Many of them must be sectioned serially to work out the small and complicated passages, glands, etc. The combined celloidin and paraffin method is most useful here, especially when the tissue is stained in bulk. In order to insure a series unbroken by stiff and curling sections, it is best to use a very weak celloidin mixture.

Many of the nidamental structures will be found lying in the passages and will

offer obstructions that are not easily surmounted. Much can be done to help this by seeing that these are removed by natural processes at the time of killing the animal. The secreted material in the cells themselves often makes the sections brittle and poor.

A better appreciation of these tissues may be obtained by observing them in action during the life of the animal. Linton and Curtis have studied them thus in transparent worms, and much interesting work has been done in this way. Such observations have been made on larger animals which were not transparent, as the cephalopod mollusks, by cutting out or exposing the parts in a freshly killed animal and watching the operations that were automatically performed until near the death of the tissue, which takes place some time after that of the individual as a whole.

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CHAPTER XXIII

PARENTAL AND EMBRYONIC NOURISHING TISSUES

Many organisms cast their ova out into the surrounding water, to develop and perpetuate the race by sheer chance and force of numbers. Others place them in "nests," and some of these even go about with the young for some time afterward, aiding them by their protection from enemies and in their search for food. The parent may even bring them food until they can procure it themselves. Still another class of parents feed the young both before and after birth with some product or part of their own body. It is with the tissues whereby they perform this latter function, that this chapter deals.

The most frequent method of supporting the young is inside the body, in contact with some surface through which the food can be passed and the waste products returned to the parent's blood. This process probably had its origin in many of the organisms which kept the young in the body for development on their egg-yolk supply. Such a method may be observed in hundreds of forms, some of them very lowly organized. Sometimes the attachment to the parent's body is maintained through the membranes of the mother's or father's mouth, as in a catfish, *Aspredo batrachus*, or the skin of the back or belly as in a toad, *Pipa Americana*. This external connection possibly furnishes the young with some benefits in its growth, possibly some small amount of fluid food.

The spiny dogfish, *Acanthias vulgaris*, is one of the animals which retain the ova in the reproductive passages during development. —The egg is very large, and when discharged from the ovary it passes into the oviduct, and in the upper part of this tube it is provided with a watery jelly layer and, later, with an outer covering of thin, transparent, flexible material which easily ruptures, but which, in other sharks and rays, is tough and heavy.

When the egg is developed so that the young animal is of some size, this outer shell breaks, and the young animal lies in the dilated lower portion of the oviduct, which thus becomes the *uterus*. Here it lies in a fluid, and the walls of the uterus become specialized to present a larger blood supply and a greater surface for exchanges between the blood and the uterine fluid.

With the naked eye, it can be seen that the entire inner surface of the uterus is thrown into a series of flat circular flaps, or papillæ, and a good-sized blood vessel can be seen passing along the edge of each flap. The true line of junction of the flap to the wall is always longitudinal, and it can be seen at a glance that this surface amplification affords more than double the original surface of the cavity.

Sections taken transversely to the uterus, and consequently to the papillæ, show the flaps in cross section with the blood vessel in the outer edge (Fig. 462, *A*). The body of the evagination is of loose connective tissue and covered with a very thin stratified epithelium. In the base

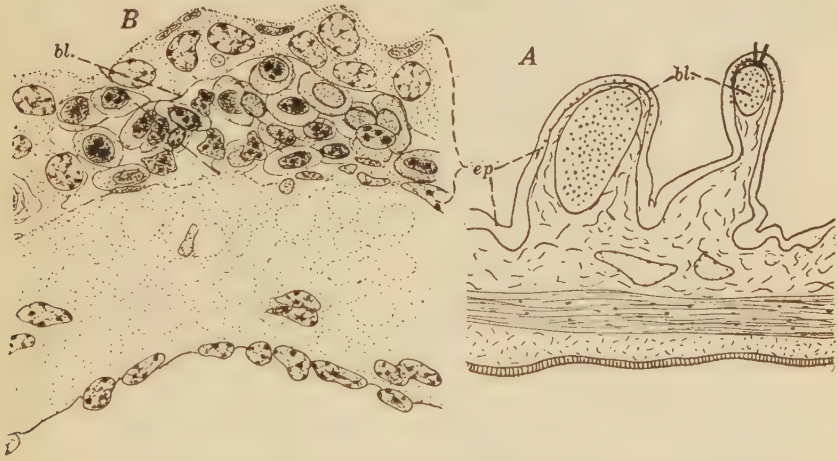

FIG. 462. — *Acanthias vulgaris; A*, Diagrammatic figure of part of a section of the wall of the pregnant oviduct (uterus), taken transversely to the length of the tube; *bl*, blood vessels; *ep*, respiratory epithelium. Lower layers are connective tissue, and longitudinal and circular muscle layers covered by a layer of epithelium (peritoneum). Black lines in top of papilla indicate the region which is shown under greater magnification in *B*. *B*, Small portion of the wall which lies between the uterine fluid and the large blood vessel on edge of a papilla; *bl*, blood capillaries in outer epithelium. *A*, × 20. *B*, × 500.

of each papilla is another vessel, usually empty or with bu a few red blood corpuscles in its lumen. It is nearly as large as the arterial loop out in the edge, and is the vein which returns the blood to the circulatory system.

The passage of the blood from the superficial loop of artery to the lower and more internal vein is the interesting structural feature of the tissue (Fig. 462, *B*). It does not flow through the body of the connective tissue, which is barely supplied with circulation, but enters a plexus of capillaries just under the stratified epithelium and so close to it that a separating blood-vessel wall is hardly discernible. The blood apparently flows in contact with, or even within, the epithelium, which is weakly stratified. These channels proceed from the top of the papilla

down to its base, where their contents are collected and carried away by the vein.

Just inside the vein, and the connective-tissue layer in which it lies, is the inner circular layer of smooth muscle which is found in all parts of the uterine wall. Outside of this, again, is the longitudinal smooth muscle layer which is covered externally by a peritoneum.

Oxygen certainly passes from the blood into the uterine fluid, and is taken into the embryonic blood by the embryonic gill filaments described in Chapter XVII. Also, urates are probably returned and excreted through the parental kidney. Carbon dioxide must also be returned. As to whether any food materials are transferred from the parental

FIG. 463. — *Myliobatis aquila*; outline of a section through the wall of the oviduct in a plane corresponding to the section in last figure. The epithelium on the papillæ is respiratory. The thicker cells lining the intermediate pockets are food secreting. (After BRINKMANN.)

blood to the embryo through the uterine fluid is doubtful. The embryo has a large yolk supply, but when finally born, it weighs much more than the new egg did. The oxygen which it has absorbed might account for part of this extra weight, also the water. On the other hand, the carbon dioxide and urea thrown off would detract from this balance. It thus remains an open question as to whether the parent furnishes its young with food other than oxygen and its original supply of yolk.

We are so fortunate in this case of doubt as to have evidence on which to base a decision. There are, among the species of elasmobranch fishes, a perfect series of stages, beginning with those that lay an egg, passing through many sharks and rays which hold the young in a uterus to develop on the yolk, as above in the dogfish, and gradually terminating

with members of the group that very clearly nourish the young with a fluid food furnished by the walls of the uterus.

We shall study, as an example of this latter histological condition, **the uterine wall of the elasmobranch fish** *Myliobatis aquila*, a ray found in the Mediterranean Sea and studied by Brinkmann. In the uterus of this fish the lining epithelium is simple, and is arranged on a series of papillæ to present a large underlying blood supply to the surface. This is just as it was arranged in *Acanthias*.

In addition to this arrangement, however, such of the epithelium as lies in the spaces between the papillæ is specialized so as to be able to take materials from the blood, and, after having elaborated them into a food material that is particularly adapted to the needs of the embryo, to discharge them into the uterine fluid in which the embryo lies, where they will be accessible for its nourishment.

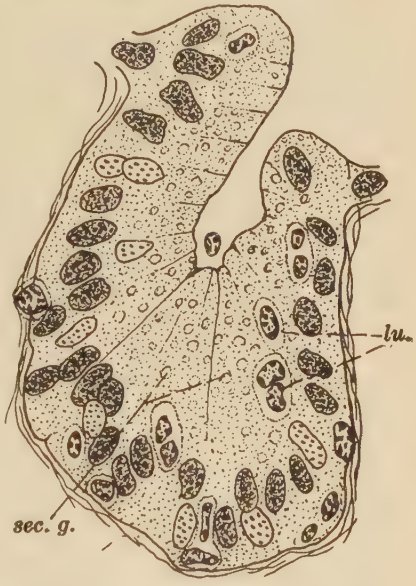

FIG. 464. *Myliobatis aquila;* a higher magnification of one of the nutritive pockets shown in the last figure. *sec.g.*, cytoplasmic secretion granules; *lu.*, leucocytes. (After BRINKMANN.)

This food material is discernible in the uterine fluid as a milky constituent, and while its chemistry and the physiology of its assimilation by the embryo have been studied, there remains much interesting work to be done. Figure 463 shows a low power, outline view of a vertical section of this uterine lining to show the relations between the respiratory and nourishing epithelium; while in Figure 464 is shown a much enlarged view of a section of one of the "pockets" of nourishing epithelium. It can be seen here that cellular elements (leucocytes), as well as the secreted materials, are passing through or between the epithelial cells. The secreted substance is observable as a series of vacuoles which are slightly larger in the distal cytoplasm than in the proximal. The writers believe, from study of Brinkmann's figure and without having seen actual sections of this material, that the two kinds of epithelial nuclei described by this author are the same, of which the lighter ones are such as have been cut by the knife in sectioning, and thus have stained more slightly.

We shall next study the extreme of this condition, as found in a

mammal, where the young start off with practically no yolk supply and depend entirely upon materials from the parent's blood for everything. We shall describe briefly this uterine surface together with the embryonic membranes by which the young animal is able to take advantage of these supplies.

The normal adult uterus, in many mammals, consists of an outer muscular layer with an inner mucous layer that is composed of a thick subepithelial connective tissue containing many lymph spaces and blood vessels. The epithelium which lines the mucous layer is a simple

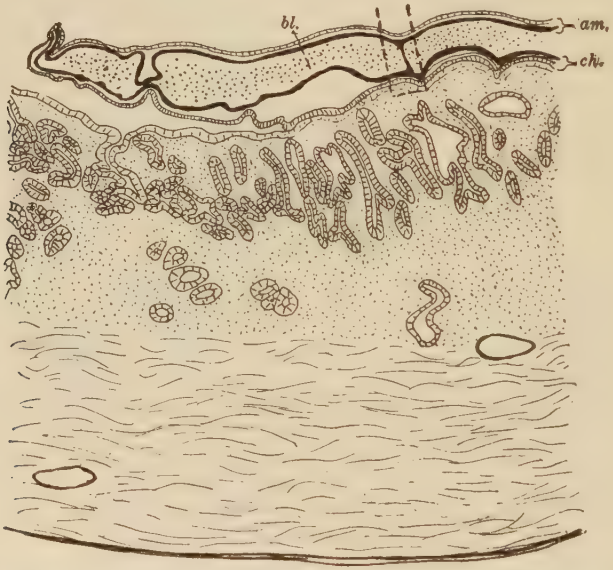

FIG. 465. — Part of a longitudinal section through the uterine wall of a cat. The fœtal membranes of a very young embryo are seen *in situ*., applied to it. *am*., amnion; *ch*., chorion; *bl*., blood. Dotted lines show region from which next section was drawn. × 45.

columnar epithelium which is invaginated into a great many simple, or sometimes branched, tubular glands that reach to the muscle layer and secrete no special fluid. The outer part of this layer with its epithelium and part of the glands are broken up and thrown off at a period called the menstrual period. This lasts a few days, after which the lost epithelium is regenerated from the remaining portions of the glands.

The fœtal membrane is composed of two layers, an outer layer covered externally with a simple epithelium, under which is some embryonic mesodermal tissue. This is the *chorion*. Also an inner layer composed likewise of a simple, but inner, epithelium resting on a mesodermal layer. This is called the *amnion*. These two layers are joined together by their mesodermal surfaces, the line of contact being marked by a very

loose connective tissue. They thus form what is practically a single mesodermal layer lined on its outer and inner surfaces with a simple epithelium. They are shown in semi-diagram in Figure 465.

This membrane is a part of the embryo's body at this time, and the fœtal circulation extends extensively into it as a plexus of small vessels in the mesodermal core. When the embryo attains a certain size, this membrane is applied by its chorionic surface to the internal epithelial surface of the uterus and forms some sort of adhesion to it. Figure 465 shows a semi-diagrammatic representation of this in the uterus of a cat, while Figure 466 shows an enlarged view of part of this same section. This relation continues to be maintained in some parts of the contact. But in others a more intimate association is formed. This occurs in different regions in different mammals. In man it is on one side of the uterus, and it is developed as follows (Fig. 467).

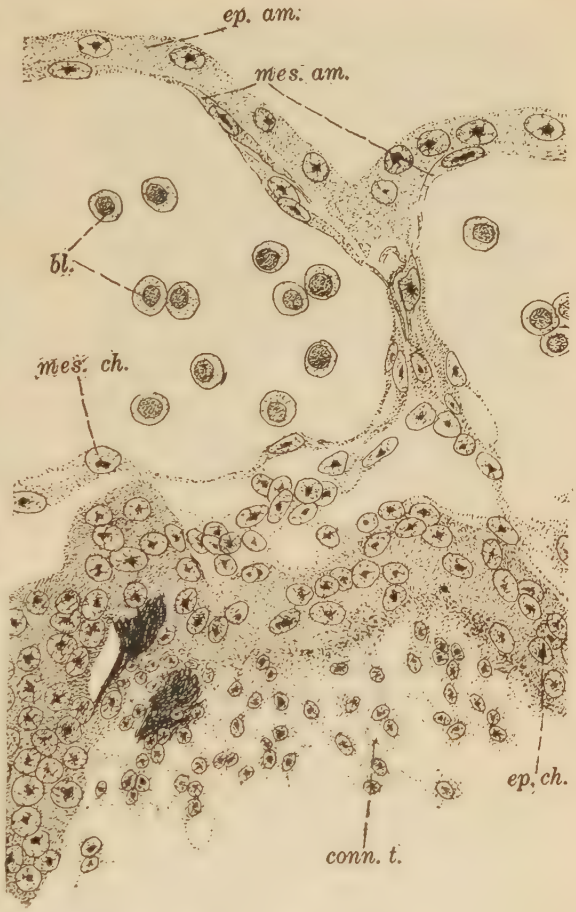

FIG. 466. — Enlargement of part of Figure 465. *ep.am.*, epidermal layer of amnion; *mes.am.*, mesodermal layer of amnion; *mes.ch.*, mesodermal layer of chorion; *ep.ch.*, epithelial layer of chorion merged with the simple epithelium that lines the uterus; *conn.t.*, connective tissue; *bl.*, blood cells of embryo. × 520.

The epithelium layer of the chorion is evaginated into a series of branching villi, which push down (outward or distally with regard to the chorion, proximally with regard to the uterine epithelium) into the mucous layer of the uterus. As the villi advance, the surface of the

uterine layer degenerates, or it may already be broken up by the menstrual process. It is destroyed and removed until only the lower parts of the gland are left, together with a residual connective-tissue layer about one third as thick as the original membrane.

FIG. 467. — Diagram of the relations of the fœtal membranes to the uterus in man at the close of pregnancy. (After SCHAPER.)

The distal ends of the uterine blood vessels are also lost in this process, and the maternal blood comes out of the free ends of the arteries and circulates in the open spaces that lie between the uterus and chorion, and among the villi. As the broken ends of the uterine veins also remain open, this blood is returned through them to the maternal circulation. Figure 467 is a diagram after Schaper to illustrate this condition.

During this development the simple epithelium on the villi has proliferated an outer layer, which differs in appearance from the original layer, which now lies in a basal portion on the connective tissue. This second and distal layer is syncytial, in that its cell boundaries are not demonstrable. It is thickest on the tips of the villi, and is incomplete nearer the chorion. Where it is thickest, it is developed into tuberosities much like those on the umbilical cord. When this membrane is torn away from the uterine wall, the blood vessels close and establish new connections between arteries and veins, the reticular tissue is thickened, and a new covering of epithelium is regenerated from the remaining portions of the glands.

It should be noticed, in the apparatus described above, that the food materials and other materials are exchanged directly from parental blood to embryonic blood, and *vice versa* through only a few thin cells. In the elasmobranch fishes, on the other hand, a uterine fluid stood as an additional agent of transfer. Also some or all of the food material probably entered the young fish's body through the digestive tract instead of directly into the blood.

The work of nourishing the offspring from the parental body is not finished even with their birth, in the mammals. It is then taken up by

the mammary glands from which the young suck an epithelial, glandular secretion called the milk. These glands consist of an embryonic invagination of the stratified epithelium into a series of aveolo-tubular glands (Fig. 468). In the acini and ducts of these invaginations, only the basal layer of cells persists as a simple epithelium which is the secreting layer of the gland.

FIG. 468. — An acinis from a functional mammary gland of the cat. The lumen is filled with the watery secretion. Several of the cells are secreting large fat droplets which are stained black with the osmic acid. × 1300.

The mode of secretion is peculiar, when we consider the fact that other oils (sebaceous and odorous) are usually produced by a degeneration

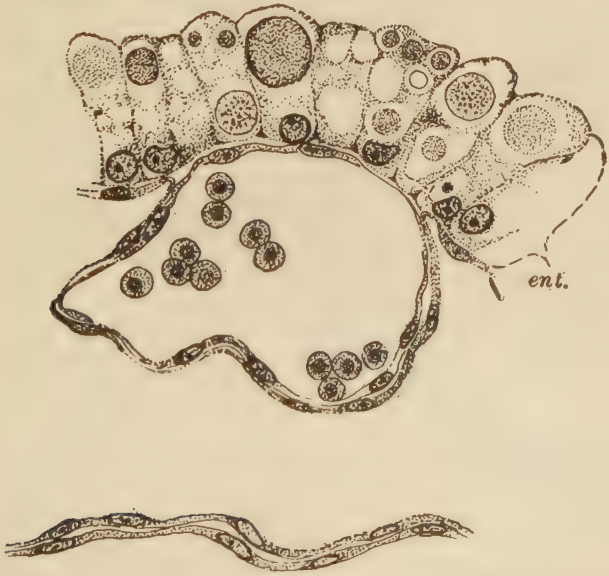

FIG. 469. — Part of the fœtal membranes of a tern, *Larix*. *ent.*, entoderm whose cells are engaged in securing nourishment from the yolk. × 435.

and disquamination of the cell. In this form of tissue the secretion is formed in the distal portion of the rather short cells, and is carried to

the surface and discharged as a droplet, the vacuole from which it came being closed behind it.

These glands are probably specializations or phylogenetic derivatives of sweat glands or of primitive glands from which the sweat glands were also derived. This is shown by their simple epithelial lining and the manner in which they produce both droplets of fat and a supply of the watery constituents of the milk.

The first part of this secretion, just after childbirth, is handled by certain lymph cells, or amœbocytes, which crawl between the cells and lie in the lumen. Here they receive the secretion and carry it out. They are called the *colostrum corpuscles*.

We must also consider here, certain embryonic membranes which are used to take food material from a yolk or store of food instead of from a maternal membrane. The parent is not concerned in this process other than by the fact that she previously furnished the yolk and that the process may take place in her body as well as outside of it. Such cases are well, but narrowly, represented by the yolk membranes of a bird, the tern, and those of a fish, the toadfish.

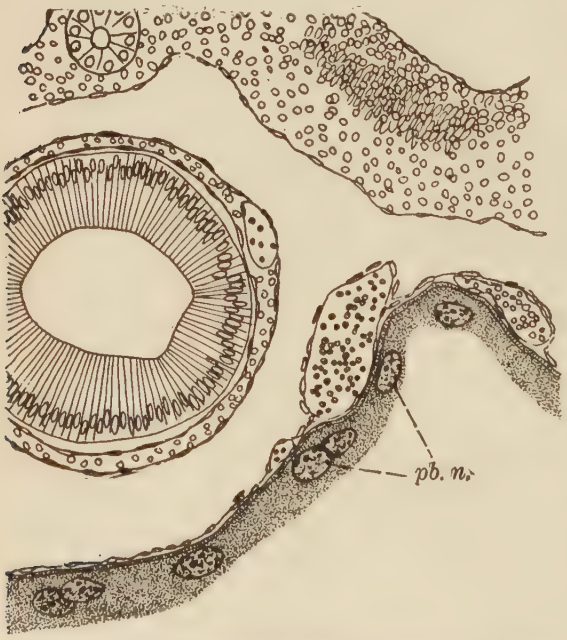

FIG. 470. — Section through an embryo of the toadfish, *Opsanus*. *pb.n.*, nuclei of the periblastic syncytium which elaborates yolk for the use of the young fish. × 250.

In the tern, the development of the embryo results in **a four-layered membrane which stretches from the body of the young bird over the yolk** (Fig. 469). The two inner layers of the four develop a system of capillary circulation. This plexus appears *de novo*, and the blood corpuscles appear as a part of the tissue that lie (?) within its walls. The outer layer is a simple epithelium derived from the ectoderm, and it has temporarily assumed the function of a respiratory membrane.

The cells of the inner layer are very much specialized and are used

to take yolk, and, having properly prepared it, to transfer it as a solution into the blood. The cells are very large, and the nucleus lies in the proximal end. The distal end shows masses of yolk which are in process of food elaboration.

The early stages of a teleost fish embryo show **a proximal layer of tissue which lies on the yolk** and is probably used at this early period to secure the nourishment from it. The layer is evidently cytoplasmic, but it shows no cell boundaries, and is therefore syncytial (Fig. 470). It contains enormous nuclei of a peculiar shape and chromatin pattern. These nuclei are known as the *parablastic nuclei*, and they multiply by amitosis. Later they are done away with, and another form of nutritive tissue is substituted.

Technic.—The nourishing tissues are easily cut in paraffin and should be studied by individual sections fixed and treated for the best cytological results. In some few cases it is desirable to understand the larger histological relations, and for this purpose bulk-stained material should be cut in celloidin. Sometimes serial sections of particular regions are necessary, and bulk-stained material, cut in medium or soft paraffin, will give the best results here.

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CHAPTER XXIV

TECHNIC

The technic of cytological and histological research has assumed formidable and intricate proportions of detail. Its fundamental ideas, however, remain the same and probably 95 per cent of the detailed modern methods are based upon, or elaborated from, these first principles.

The student is advised to read and digest the following general outline of the principles of technic, and then execute the several complete schedules. After this, if he wishes to further master technic, he should prepare for study the specimens which appear in each chapter, following the outline directions given in special cases, and these will afford him a sufficiently varied and extensive practice for all purposes. The "Microscopist's Vade Mecum," by Lee, should be at hand and referred to in this connection.

The methods are in all cases the best that the writers have actually had experience with. They are not picked out with reference to an inexperienced student and a poorly equipped laboratory, but call for the best of instruments and reagents, and an experienced instructor. In different hands, other methods will sometimes be found to do the work better in certain cases, and the examples found below are offered as a convenience or a starting-point.

GENERAL OUTLINE

Most tissues cannot be studied in a fresh condition because of several obstacles; they are too thick and cannot be cut into thin slices on account of their texture. Besides, their parts are nearly of one common color and refractive index, which makes the structure indistinct at the best, and, lastly, they will soon decay or dry and are not permanent.

So our technic is devised to get slices thin enough to see, to change the color and refractive index to more favorable conditions, and to make more or less permanent preparations.

The majority of tissues containing much protoplasm must first be *fixed*, which means *killed* by some medium that leaves them in a con-

dition that is as near as possible to life, and that will withstand change for some little time. *Fixation* results in a whitened appearance and a firmness, or even a considerable hardening, of the tissue. The fixatives used are usually mineral acids and salts, organic acids, and sometimes heat. A natural death of the tissue would result in its speedy disorganization.

The second step consists of the use of fluids that will further *harden* and preserve the tissue, and at the same time will prepare the way for subsequent treatment by removing all unnecessary or injurious substances. The fixatives, especially, must be removed to avoid artifacts, due to crystallization, etc. Alcohol is a fluid admirably adapted to furnish these two results, especially when water is first used, in some cases, to remove substances insoluble in alcohol. Lastly, the tissue must be *dehydrated* for purposes we shall soon see, and here again alcohol is the best reagent.

This decides the use of alcohol as fulfilling all purposes, and it is used in gradually increasing strengths to prevent too rapid osmosis and consequent distortion. Sometimes additional substances are used in the alcohol to help remove the fixative, as iodide of potassium after mercury bichloride.

The third step is decided by our ultimate object of getting the piece of tissue embedded in paraffin (or in celloidin) so that it may be cut in thin sections. Paraffin will not mix with alcohol, so an intermediate fluid, some oil that will mix with both alcohol and with melted paraffin, is employed and substituted for the alcohol. This step is known as *clearing*. Common clearing reagents are cedar oil, toluol, xylol, etc. Chloroform is also a valuable clearing reagent.

No obstacle now exists to prevent melted *paraffin* from being substituted for the clearing reagent. This is done in an oven just warm enough to keep the paraffin melted, and, when infiltrated, the mass should be poured out into a paper box, the bit of tissue oriented, and the mass cooled. When the block is cooled, we find that the tissue is completely embedded in, and infiltrated by, the paraffin. The mass, which is hard, can be cut with a knife into very thin sections without injuring the tissue, or altering its structure or the relative position of its parts.

The sections should now be caused to adhere to a glass microscope slide, the paraffin melted off with xylol or other clearing reagent, and the specimen then freed from the clearing reagent by alcohol and from alcohol by water. The proper dyes will now stain the cell and its organs with a differential stain that should enable all parts to be seen. When stained, the slide must be dehydrated again, then cleared, and lastly a drop of balsam placed on the section, which is then covered with a thin cover glass.

This is the best way known in most cases of seeing an accurate picture of the details of structure in a tissue. Good results can be attained by staining the tissue after fixation, and before it is dehydrated. In that case, when the sections are cut and mounted on the slide, they may be at once freed from paraffin and mounted in the balsam. This way of staining *in toto* or *in bulk*, although shorter, seldom shows the detail and accuracy of sections stained on the slide. It is a valuable method, nevertheless.

In case embedding in celloidin is decided upon, the material is dehydrated and immersed in a solution of *celloidin* or photoxylin dissolved in ether or absolute alcohol, or, as is more commonly done, in a mixture of both. After a long soaking to allow of a good infiltration, the solution is thickened by evaporation or the addition of a stronger solution, when the mass, now nearly solid, may be hardened in chloroform or its vapor, and then cut into sections which are not as thin as paraffin sections, but usually have a peculiar beauty of their own, due to their not having been subjected to the shrinking action of cooling paraffin. As in the paraffin method, these sections can be stained separately before mounting, or the material can be previously stained in bulk, which is perhaps better for the celloidin method, and permits of immediate mounting when cut. Otherwise the sections are stained as "free sections" or, in rare cases, on the slide.

The student should consult Lee for the cutting of sections of bone, etc., and other exceptional cases.

First Example

Secure a salamander (*Necturus* preferred).

A. Cut portions of liver into cubes of 10 to 15 mm. or less.	Large enough for a view of its structure. Small enough for penetration of fixative and other processes.
Place pieces in a saturated solution of corrosive sublimate, in water, to which 5 per cent of glacial acetic acid has been added. Allow to remain one hour to two hours.	To fix.
B. Rinse and move to 70 per cent alcohol for one hour. Change the alcohol once. Place in 25 cc. or 50 cc. of 80 per cent alcohol for one or two hours more.	To harden, and to remove such sublimate and mercury compounds as are soluble in water and alcohol.

Add 1 cc. of solution of iodine ($\frac{1}{4}$ per cent) and potassium iodide (1 per cent) in water. Soak in this for three hours or more. Place in clean alcohol (80 per cent) for one or more hours. Repeat the treatment with potassium iodide and then remove it by soaking it again in 80 per cent alcohol.	To prevent the crystallization of the sublimate and to remove certain compounds of mercury that would otherwise spoil the specimen.
C. Place in 95 per cent alcohol for one hour, drain on blotting paper, and place in absolute (99 per cent) alcohol for one or more hours. Change once.	To dehydrate.
D. Place in a small quantity of xylol or toluol. If a milky color appears and persists, dehydration is not complete and the specimen should be returned to *fresh* absolute alcohol for one half hour and then back to xylol. The specimen will now become translucent or even transparent and is then ready for embedding.	To replace the alcohol with a substance that will mix with melted paraffin.
E. Place in melted paraffin of 52–56 melting point in an *open* dish. Keep in a water bath which is slightly warmer than the melting point of the paraffin. Change the paraffin once or twice, and in from one to two hours the specimen will be properly *infiltrated and ready to embed.*	To infiltrate with, and embed the specimen in paraffin.
F. Embed in a paper box. See that marks on the box show in which plane the sections are to be cut. It is well to adopt some regular position and try to embed so that the sections should always be cut from one surface or side of the box, preferably the under surface. Cut sections, on a rotary microtome, 5 to 10 microns thick. Attach to a *clean* slide by placing several drops of water on the slide and floating the sections on, taking care to remove all air bubbles caught under the folds of the sections. *Heat on the top* of water bath to a point not over 15° to 10° below the melting point of the paraffin used. If the section melts at any point, such part will not afterwards adhere. When the section has straightened out under this gentle heat, allow the water to drain from under it and arrange it to suit with a needle or scalpel; never work with one tool, but have one pointed instrument in each hand so that if the section sticks to one instrument the other can be used to release it. Surface tension will make or mar the arrangement according as it is understood and used, or ignored and fought with. Thus two rows of flattened sections, freely floating, may absolutely refuse to be drawn together to make room for a third row outside of them. If now the point of a scalpel be drawn between them several times to break up the invisible film of paraffin on the water, they will come together themselves. In fact, they could not be kept apart.	Instructor must supervise this work.

When arranged, place the slides in a dry, well-ventilated place for six or more hours to dry out completely. The sections will then adhere through any other process. When the fixative contains chromic acid or any of its compounds, egg fixative must be very slightly smeared on the slide before placing the water and sections

upon it. It is perhaps best to make a practice of using the egg fixative in all cases.

The slides can now be stored for some time in dust-proof boxes. It is best, however, to proceed soon after this to stain and mount them. We shall continue our description of a particular example.

G. Place the slide in xylol for three minutes.	To remove the paraffin.
Rinse it in absolute alcohol and then in 95 per cent alcohol. It is now ready to stain.	To remove the xylol.
H. Place the slide in a 1 per cent solution of iron alum.	To mordaunt it or prepare it for the stain.

Rinse in distilled water and transfer to a one fourth of 1 per cent solution of hæmatoxylin in distilled water. Experience will tell how long to stain. From six to twelve hours is usually right. The longer stain will bring out the achromatic and cytoplasmic structures best.

When stained, the slides must be rinsed and returned to a somewhat weaker solution of the same iron alum that was used to mordaunt them. They must be watched while the color is extracted and had best be frequently taken out, rinsed, and examined with an old microscope to see that the proper reaction has taken place. They will look darker when finally mounted than when thus examined in water. The stained slides should now be washed in gently running tap-water or in a number of changes of fresh water to remove *all* traces of the iron alum. They are then ready for —

Mounting

I. Place the slides in 95 per cent alcohol for a few minutes. Transfer them to absolute alcohol for about five minutes.	To remove *all* water.
Place them in two successive baths of xylol.	To remove all alcohol and replace it with an oil that will mix with balsam.

Drain off excess of xylol and promptly place a drop of some *good, acid-free* balsam solution on the specimen, which *should still be wet with xylol. It must at no time be allowed to dry.* Use Grübler's damar balsam dissolved in xylol. The cover glass, of number one glass, should now be placed in position *without pressing on its top when in place*, and the slide laid flat in a warm place. Any air bubbles which happen to be inclosed will find their own way out in a few days, if the proper amount of balsam is present. Balsam may be added in small drops at the edge of the cover. The balsam should be diluted, if too thick, with pure, fresh xylol.

The above is a single concrete example. We shall suggest as a desirable variation the following —

Second Example

AA. Fix bits of the same tissue in Flemming's solution of —

1 per cent chromic acid	15 parts
2 per cent osmic acid	4 parts
Glacial acetic acid	1 part

Use smaller or thinner portions of tissue if possible and place in a rather small quantity of the fluid. Do not change the fluid. Fix for twenty-four hours to ten days.

BB. Same as (*B*), except that the tissue should be washed for a time in clean tap or distilled water and the iodide treatment dispensed with. Subsequent steps are the same.

Third Example

Another variation that should be tried is as follows: Treat tissue according to (*A*) and (*B*).

HH. Stain for twenty-four hours in a solution of borax carmine. Decolorize for twelve hours, with frequent changes, in 70 per cent alcohol to which hydrochloric is added (6 drops to each 100 cc.).

Then proceed as in (*C*), (*D*), (*E*), (*F*), and (*G*), except that the alcohols should be omitted in this latter step. As the sections are already stained, they may then be mounted as in (*I*), except that the first treatment (with alcohol) may be omitted as the sections are already free of water.

Fourth Example

Celloidin embedding should now be practiced as follows. Proceed, with the same tissue, as in (*A*), (*B*), (*HH*), and (*C*).

J. Place in a quantity of celloidin dissolved in equal parts of ether and absolute alcohol. The amount of celloidin should be from 1 to 2 per cent. This should be in a well-stopped bottle, and a long treatment of days or even weeks is beneficial. Forty-eight hours will do. The strength of the celloidin solution should be increased to 6 per cent, and finally the object should be placed on the end of a cork surrounded with a covering of thick celloidin solution, and as it becomes firm on the surface, plunged into pure chloroform (on the carrier) for an hour or more, to harden.

K. When hard, it should remain in a mixture of *cedar oil* (or cedarwood oil) and chloroform, equal parts, for another hour, when the whitened celloidin will become clear. Sections may now be cut with a knife wet in the chloroform-cedar oil mixture. A soft brush must be used to keep the knife flooded, and chloroform must often be added to compensate for evaporation. When the cedar oil is in excess, the celloidin softens or even melts.

L. The free sections may be floated on a slide with a brush, drained, and at once mounted in balsam. They are already dehydrated and cleared and need only the balsam and a cover glass.

One more method should be carried out in the concrete as follows in this —

Fifth Example

Prepare a bit of rat testis by fixation, etc., as in (*A*), (*B*), (*III*), and then restain it in Mayer's hæmalum (see Lee) for twelve to eighteen hours. Extract the stain for six to ten hours in 1 per cent alum water (common alum). Dehydrate (*C*), infiltrate with celloidin (*J*), and clear as in (*K*), except that the specimen is free and not placed on a cork or other carrier.

Now embed (*F*) and cut thin sections; these may be either handled free (*L*) by dissolving the paraffin or floated with water on the slide (*G*) and mounted, or even fastened to the slide and restained before mounting if the stain has proved unsatisfactory.

A favorite method in medical work, where fairly thick sections from the tissues of mammals are desired, is to infiltrate unstained material with celloidin, harden in

moderately strong alcohol (80 per cent), cut, stain with hæmatoxylin and eosin, and then clear and mount.

The preceding methods are sufficient for most work. Some of the steps, as *fixation* and *staining*, have hundreds of variations. A few of these variations have been mentioned after the different chapters in regard to some of the more difficult tissues. For methylene blue staining *intra vitem*, and the various silver and gold methods and others, see Lee.

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